Institutional Animal Care and Use Committee (IACUC)

Standard Operating Procedures

1 Institutional Animal Care and Use Committee (IACUC)

1.1 Policy

The State University of New York at Stony Brook fosters a research environment that promotes the respect for the welfare and safety of animals used in research. All use of vertebrate animals in research, teaching and testing is regulated by the Institutional Animal Care and Use Committee (IACUC).

1.2 Mission

The mission of the IACUC is to:

- Protect animals, employees, staff, students, and the public involving the use of animals in research
- Perform required reviews for the use of animals in research

1.3 Definitions

Adverse Event
"Adverse Event" means any untoward medical occurrence associated with the use of a drug or device in animals, whether or not considered drug related. It does not include an **adverse event** or suspected **adverse reaction** that, had it occurred in a more severe form, might have caused death.

Aseptic Surgical Procedures
"Aseptic Surgical Procedures" means surgery performed using procedures that limit microbial contamination so that significant infection or suppuration does not occur.

Disinfection
"Disinfection" means the chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.

Major Surgery
"Major Surgery" means any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for producing permanent physical or physiological impairment; and/or any procedure associated with orthopedics or extensive tissue dissection or transection.

Minor Surgery
"Minor Surgery" means any surgical intervention that neither penetrates and exposes a body cavity nor produces permanent impairment of physical or physiologic function. Examples are superficial vascular cut down, and percutaneous biopsy.

Sterilization
"Sterilization" means the process whereby all viable microorganisms are eliminated or destroyed. The criterion of sterilization is the failure of organisms to grow if a growth supporting medium is supplied.

1.4 IACUC Organization and Operation

The IACUC is administered by the Office of Research Compliance (ORC), which also monitors compliance and promulgates policies and procedures to ensure that the IACUC membership is duly constituted in accordance with federal regulations.

There is a minimum of 5 members on the IACUC. The IACUC chair is selected from the IACUC members by the Vice President for Research. The IACUC chair signs all minutes and reports for the IACUC.

Applications (i.e., Curriculum Vitae) for new members are submitted to the IACUC coordinator and brought to the IACUC meeting for review. The IACUC members determine if the individual meets the requirements for membership on the committee. Once the review of the CV is complete, the individual is notified regarding their status as a potential IACUC member. The potential new member may then observe an IACUC meeting.

Changes in membership and applications for new members must be submitted as soon as vacancies occur on the committee. A current, curriculum vitae for each committee member must be received by the IACUC coordinator every two years.

The IACUC meets at least once per month, or more frequently, at the discretion of the Chair. No member may vote on a protocol in which he/she is an investigator. Members who do not attend at least 50% of meetings in a calendar year may be dropped from membership.

Meetings require a quorum to occur (more than 50% of the members). Protocols that involve special populations such as wildlife, should be reviewed by a specialist with expertise in that area as a consultant. The IACUC members vote on each protocol reviewed at the meeting. A majority of the members must vote to approve the application for the application to be approved. A member who has any involvement in a protocol or some other conflict of interest must abstain from voting on it.

Members are under strict requirements to maintain confidentiality regarding service on the IACUC. This requirement remains in full force during the entire term of service with the IACUC and continues in effect after such affiliation terminates.

Decisions of the committee are documented in the minutes. Minutes are confidential documents and are not available outside of committee members. The minutes are

drafted by the IACUC Coordinator. The minutes will include a listing of the members who were present for the meeting, voting results and outcome of review. The minutes will include the numerical results of votes on protocols. Comments from the protocol reviews will be communicated to the Principal Investigator by the IACUC Coordinator.

It is the responsibility of the Principal Investigator to report all adverse events. If the IACUC receives an adverse event report, the IACUC will review the adverse event at a convened meeting. If it is determined that the adverse event is possibly related to the study procedure, the IACUC has the responsibility for reporting the event to the regulatory agencies.

1.5 General Guidelines for Working with Animals in Stony Brook University Laboratories

These guidelines are intended for all investigators conducting animal procedures outside of the Division of Laboratory Animal Resources (DLAR) housing facilities.

- Animals should be transported from DLAR to the laboratory using freight elevators (when & where available) and always by using a method that shields the cages from being viewed by the general public (i.e. enclosed transport carts, kraft paper wrapping).
- Adequate supplies of food and water should be placed in the cage prior to transportation to the laboratory. During the transport the water bottles should be inverted to prevent spills. Remember to re-invert the H2O bottle so water is available to the animal after transport.
- If the animals will be returned to the DLAR, record the number of animals and the date they are removed from the facility on the census sheet in the Transfer column. If the animals will be euthanized in the lab, indicate the number of animals and the date in the Euthanized column.
- If animals will be housed overnight or longer than **12 hours** outside of the facility a strong scientific justification is required and must be approved by the.
- If animals are to be housed overnight or longer than 12 hours, a **Laboratory Housing Log Sheet** must be maintained in the lab to record the monitoring and husbandry performed each day. The forms must be available for inspection by the IACUC when they do their rounds. Animals must be checked daily, including weekends. If they remain long enough to require cage changing (3 days for multi-housed animals, 7 days for single housed animals), clean cages, bottles and fresh food should be obtained from DLAR and brought up to the laboratory and the used cages brought back down to the dirty side of the cage-wash area. Log forms are available for downloading: https://osa.stonybrookmedicine.edu/research-core-facilities/dlar/forms
- If housing for 24 hours or greater the facilities need to be inspected by DLAR staff prior to housing animals in the laboratory facility.

- If animals are to be housed overnight or longer than 12 hours, they must be maintained in an area with temperatures in a range recommended for the specific species and with lights that can be turned off at night.
- If survival surgical procedures are being conducted, the surgical area, the animal, the surgeon, and the instruments must all be properly prepared and aseptic (sterile) and proper surgical technique must be followed.
- The currently approved IACUC protocol covering the animal activities being conducted in the laboratory should be available for quick access/reference by lab personnel.
- Appropriate Material Safety Data Sheet (MSDS) forms for hazardous chemical and biological agents being used for animal studies in the laboratory must be posted and visible for both workers and emergency personnel.
- Animals returning to the DLAR must check with DLAR regarding what the rooms to which they will go.

1.6 Blood Sampling Methods of Rodents

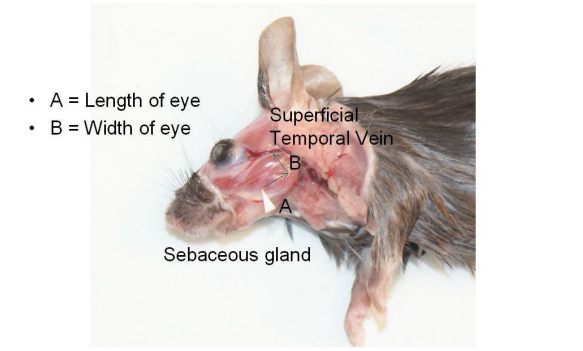
Note: The bleeding method(s) to be used must be described in the protocol narrative.

For survival procedures, the maximum amount of blood that may be withdrawn at one time is 10% of the animal's blood volume. This can be done weekly. The blood volume in rats and mice is 7% of their body weight (e.g., 0.21 ml's from a 30gm adult mouse and 2.1 ml's from a 300gm adult rat).

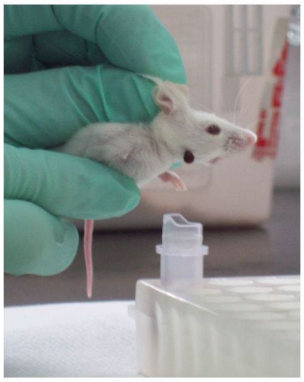
There are multiple vessels that can be used to collect blood from rodents including the superficial temporal vein (SVT) of the face; the saphenous vein of the leg, the lateral tail veins, tail tip transection, the retro-orbital plexus behind the eye, and the heart. The SVT and saphenous require only physical restraint of the animal and should be the first methods considered for sampling. The lateral tail vein or tail tip transection is typically only indicated for repeat bleeds requiring small samples (i.e. glucose tolerance testing). If performing a transection, the first sample requires anesthesia in adult rodents.

Subsequent samples can be obtained by removing the initial scab. The retro-orbital method must be done under anesthesia and should only be used when precise sample timing is needed (i.e. pharmacokinetic studies), when very large "survival" samples are needed, or when the peripheral vessels are being used for infusion. The cardiac puncture method is only done as a terminal procedure.

1.6.1 Superficial Temporal Vein Sampling



For the STV (sometimes referred to incorrectly as the facial or submandibular vein), sampling is usually done with the Golden Rod Animal Lancet manufactured by MEDIpoint. The 4 mm length is sufficient for most mice. Use the 5 mm or the 5.5 mm length for large strains such as MRLs, for very obese mice, or for mice over 6 months. Always use the shortest point necessary to draw the amount of blood you need. Sampling is limited to adult mice.



1) The mouse is held so the face is relaxed and the skin loose.
2) The sebaceous gland is located and the lancet tip moved back one ‘eye length’ and up one ‘eye width’.
3) The sterile lancet is inserted with consistent, swift pressure to the base of the lancet tip and quickly withdrawn. It should not be a jabbing motion but a smooth, controlled insertion. Up to 500 ul can be collected by this method.

1.6.2 Saphenous Vein Sampling

Blood is collected from the lateral saphenous vein which runs dorsally and then laterally over the tarsal joint. The site is shaved either with a scalpel blade, using a gentle stroking motion in the direction of the hairs and holding the blade almost parallel to the

skin to avoid cutting it, or with electric clippers. Shaving need only be repeated as the hair grows.

The conscious rodent should be restrained either manually or using a restraint tube. This can cause stress and therefore the duration of restraint should be minimized. Where a restraint tube is used, it should be appropriate for the size of the mouse.

1) To collect blood, the hind leg should be immobilized in the extended position by applying gentle downward pressure immediately above the knee joint. This stretches the skin over the ankle, making it easier to shave, and immobilizes the saphenous vein. An aseptic technique should be used. Anesthesia is not necessary but may be used on welfare grounds for animals that are difficult to hold.
2) A 27g or 25g needle is used to puncture the vessel. The number of attempts to take a blood sample should be minimized (no more than three needle sticks in any one attempt). Blood is collected by capillary action into a hematocrit tube or passively into a tube.
3) Blood flow can be stopped by gentle finger pressure over the puncture site, or simple relaxation of the operator's grip on the animal's leg. Animals should not be returned to their cage before the blood flow has stopped.

If large or repeated samples are needed, then temporary or surgical cannulation should be considered. The scab or blood clot is removed for multiple samples. Total sample size is typically limited to 200 ul. Petroleum jelly can be used on the skin to promote the blood to bead up, but it may interfere with some assays. The clot/scab can be gently removed for repeated serial sampling of small volumes.
Mice may show temporary favoring of the limb following sampling from the saphenous vein.

1.6.3 Tail Vein Sampling

Tail vein sampling is suitable for all strains but is more difficult in black or pigmented mice. For competent individuals, it is quick and simple to perform. This technique may require the animals to be warmed in order to dilate the blood vessel prior to taking the sample. The lateral tail vein is usually used and 50 ul to 200 ul of blood can be obtained per sample depending on the size of the animal and specific requirements. The tail may need to be wiped with 70% alcohol in order to see the blood vessel. The number of attempts to take a blood sample should be minimized (no more than three needle sticks in any one attempt) and sufficient time should be given for the tail to recover between blood sampling sessions. Alternate sides of the tail should be used and successive needle punctures moved towards the tail base.


1) The animal is placed in a restrainer and warmed with a heat lamp or heating blanket.
2) The lateral tail vessel is located and either punctured using a 25g-27g needle or a catheter is inserted into the vessel.
3) After the sample is taken, blood flow should be stopped by applying finger pressure on the soft tissue. A finger should be placed at the blood sampling site for approximately 30 seconds before the animal is returned to its cage.

1.6.4 Tail Transection Sampling

Snipping the tail surgically is a relatively crude method of blood sampling and should be avoided where possible, or only undertaken under anesthesia, because of the potential pain and permanent damage to the tail. The saphenous vein and tail vein are more appropriate routes of sampling for most studies and strains.

Where other routes cannot be used, tail snipping should be performed aseptically, taking no more than 1 mm from the tip of the tail using a new scalpel blade. A local anesthetic cream (e.g. EMLA cream) can be applied to the site 30 minutes prior to blood sampling. For mice over the age of 21 days, a general anesthetic must be used.
Unless doing a terminal procedure, approximately 10 ul can be obtained per sample by gently 'milking' the tail.

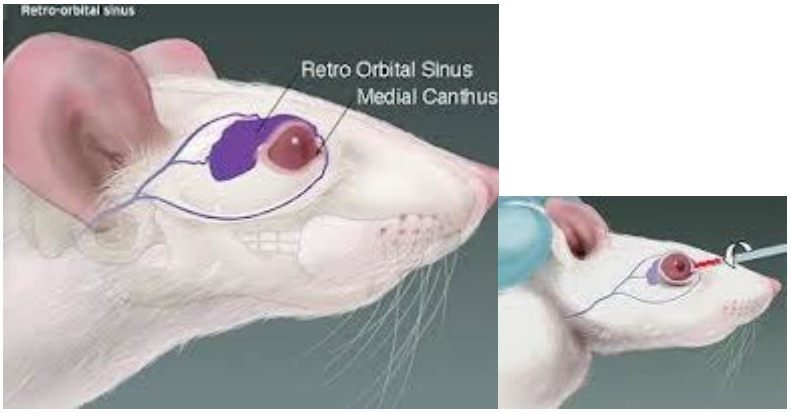
Normally no more than a maximum of four samples should be taken in any 24 - hour period. Where multiple samples are collected, this should be done by removing the scab or blood clot from the tail tip. Blood flow can be stopped by dabbing the tail tip with a clean tissue and bleeding usually stops immediately.

1.6.5 Retro-orbital Sampling (discouraged and requires justification in protocol)

Also referred to as peri-orbital, posterior-orbital and orbital venous sinus bleeding. Retro-orbital bleeding should typically be performed as a **terminal** procedure. It should only be used with recovery in rare circumstances with exceptional scientific justification (e.g. where a large blood volume is necessary, precise sample timing is needed, or

where peripheral veins are used for dosing) because of its potential impact on animal welfare.

Where its use is unavoidable, retro-orbital bleeding should only be used under general anesthesia. Because of the severity of the adverse effects that can occur with this technique, even in skilled hands, it is essential that it is conducted only by staff members competent (practiced) in the technique.



Retro-orbital Bleeding Procedure

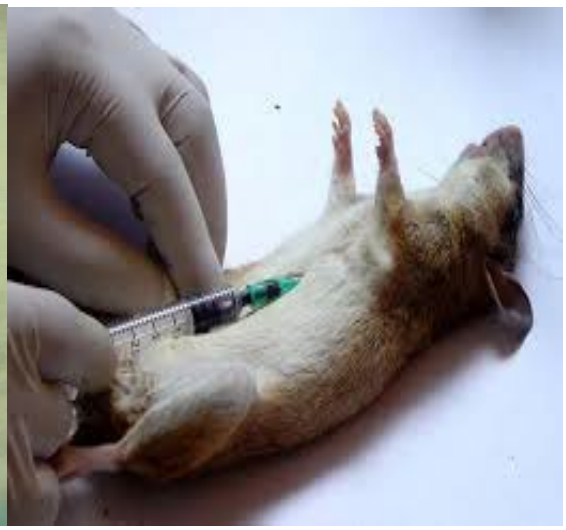
1) Standard heparinized or non-heparinized micro-hematocrit capillary tubes can be used for blood collection.
2) The animal is held by the back of the neck and the loose skin of the head is tightened with the thumb and middle finger.
3) The tip of the capillary tube is placed at the medial canthus of the eye under the nictitating membrane
4) A short thrust past the eyeball will enter the slightly resistant membrane of the sinus. The eyeball itself remains uninjured.
5) As soon as the sinus is punctured, blood enters the tubing by capillary action. It may be helpful to retract the tube to facilitate blood flow.
6) When the allowable amount of blood is collected (see additional information for parameters), the tube is withdrawn and slight pressure with a piece of gauze on the eyeball is used to prevent further bleeding.

1.6.6 Cardiac puncture sampling

Cardiac puncture is a suitable technique to obtain a single, large, good quality sample from a euthanized mouse or a mouse under deep terminal anesthesia if coagulation parameters, a separate arterial or venous sample or cardiac histology are not required. It is appropriate for all strains of mouse.

The animal is either anesthetized or asphyxiated with CO_2 prior to sampling. Anywhere from 0.1 - 1 ml of blood can be obtained depending on the size of the mouse and whether the heart is beating. Blood samples are taken from the heart, preferably the ventricle, which can be accessed either via the left side of the chest, through the diaphragm, from the top of the sternum or by performing a thoracotomy. Blood should be withdrawn slowly to prevent the heart collapsing.

Cardiac puncture should not be used if the peritoneum needs to be lavaged to harvest cells, as this technique can cause blood to escape into the peritoneal cavity.



1.6.7 Anesthesia

Several of the methods above (tail transection, retro-orbital bleeding, cardiac puncture) require the use of anesthesia:

1) Anesthetic Agents - animals should be anesthetized with one of the following agents:

Mice	Ketamine (90 mg/kg) and Xylazine (10 mg/kg), IP Pentobarbital (50mg/kg), IP Isoflurane (1-5%) or open drop
Rats	Ketamine (75 mg/kg) and Xylazine 10 mg/kg), IP Pentobarbital (50 mg/kg), IP Isoflurane (1-5%) or open drop

2) Use of any other anesthetic agents must be identified in the IACUC application.

1.6.7.1 Monitoring

Prior to and during the procedure the following parameters must be monitored at a minimum of 5-minute intervals:

- Respiratory rate
- Response to noxious stimulus
- Spontaneous movement

1.6.7.2 Recovery Monitoring

1) During recovery from anesthesia, the following clinical parameters must be monitored at a minimum of 15-minute intervals until the animal is ambulatory.
   - Respiratory rate
   - Movement
   - Ability to maintain sternal recumbency
2) To protect the animal from hypothermia they should be placed on a water re-circulating heating blanket, or covered well, to conserve body temperature. Animal should never be placed on metal surfaces.
3) It is estimated that animals will recover within 30-60 minutes postoperatively.

1.6.7.3 Analgesia

Analgesia is not required for these procedures, but the use of topical anesthetic (Proparacaine – 1 drop per eye for retro-orbital) or EMLA cream on the tail (transection sampling) is encouraged to decrease pain post-procedurally.

1.6.8 Blood Collection Parameters

1) For survival procedures, the maximum amount of blood that may be withdrawn at one time is 10% of the animal's blood volume. The blood volume in rats and mice is

7% of their body wt (e.g., 0.21 ml's from a 30gm adult mouse and 2.1 ml's from a 300gm adult rat).

2) If a project requires a greater volume of blood withdrawal, more frequent bleeds or an increase in the total number of bleeds, any change in the parameters listed must be scientifically justified in the IACUC application. It is possible to sample up to 20% of the blood volume if adequate (i.e., 2 weeks or more) recovery time is given.
3) For the retro-orbital method, blood can only be collected once per week from one eye. Subsequent bleeds should use alternate eyes. The maximum number of bleeds for each animal is two bleeds per eye (four bleeds total).

1.6.9 Adverse Effects

Potential adverse effects from these blood collecting procedures include:

- Anesthetic related respiratory distress
- Restraint related respiratory distress
- Eye infection and/or peri-orbital swelling, redness and/or hematoma formation (retro-orbital)
- Anemia
- Hematoma formation

1.6.10 Clinical Monitoring and Management

1) Animals should be monitored at least twice weekly after each bleed.
2) If adverse effects are seen, the investigator should consult immediately with the DLAR veterinary staff regarding treatment options. If animals have acute adverse reactions to the anesthetic agents (respiratory distress and/or lack of recovery), they must be euthanized immediately.

1.6.11 Early Endpoints

Retro-orbital sampling - animals should be euthanized if the eyeball is acutely damaged, if treatment of an injured/infected eye is unsuccessful, and/or if bilateral blindness occurs.

All methods – hematoma formation that interferes with ability to eat, drink, locomote.

1.7 Tail/Toe Biopsy in Mice (for Genetic Analys)

1.7.1 Tail Biopsy

1) Mice may be identified by analysis of genomic DNA obtained from a tail biopsy. Sufficient DNA for PCR, Southern, and dot blot analysis can be obtained from a fragment of the distal portion of the tail (depending on age of animal). The tail biopsy can be obtained by a trained investigator from mice under 3 weeks of age

without anesthesia. If the mouse is older than 3 weeks or a larger section of tail is required, an appropriate anesthetic agent should be used.

2) Isoflurane is the preferred anesthetic agent and should be used whenever possible. If injectable anesthetic is used, it must be dosed according to body weight. For mice, ketamine (90-120 mg/kg) and Xylazine (5 - 10 mg/kg) can be injected IP. The recovery time for injectable anesthesia is much longer than with isoflurane.
3) When the animal is sufficiently anesthetized, 1-6 mm max (1-3 mm up to 3 wks old; 3-6mm for over 3 weeks old) of the tip of the tail is removed using a new scalpel blade or sharp sterile scissors.
4) Hemostasis can be achieved using a sterile gauze pad to apply direct pressure to the wound.
5) Only one biopsy is allowed for each animal. Additional biopsies require specific justification previously approved by the IACUC. A maximum of 6mm is allowed even if repeat or multiple biopsies are needed to be obtained. Anything greater than that requires scientific justification and approval by the IACUC prior to taking them.

1.7.2 Anesthesia Monitoring for Tail Biopsy

Prior to the tail biopsy, the following parameters must be monitored, at a minimum, to ensure adequate anesthesia:

- Respiratory rate
- Response to noxious stimulus (ie. Toe pinch)
- Spontaneous movement

1.7.3 Anesthesia Recovery Monitoring for Tail Biopsy

1) During recovery from anesthesia, the following clinical parameters must be monitored a minimum of every 15 minutes (or sooner), until the animal is ambulatory:
    - Respiratory rate
    - Movement
    - Ability to maintain sternal recumbency
2) It is estimated animals will recover within 3-5 minutes from isoflurane or 30-60 minutes from injectable anesthetic.
3) For injectable anesthesia or if recovery is expected to be longer than 3-5 minutes, to prevent hypothermia animals should never be placed on metal surfaces- place animals on a water re-circulating heating blanket or wrap them in a paper towel (while still allowing visible monitoring) to conserve body temperature.

1.7.4 Recovery Monitoring for Tail Biopsy

All pups must be observed for at least 24 hours post-biopsy for lethargy or failure to thrive (in rodents <10 days old, by monitoring the milk spot in their abdomen to determine if they are feeding appropriately, in rodents >10 days old, by monitoring their

mentation, hydration and presence within the nest). Pups that fail to recover or are rejected by their mother must be euthanized and removed from the study.

1.7.5 Toe Clipping

Toe Clipping Procedure for genotyping: In cases when house animals of mixed genotype are grouped together, permanent marking of the genotype becomes necessary. Moreover, breeder animals maintained for 6 months or longer may require clear permanent identification. The procedure is to be used in mouse pups during the first week of age.

1) During the procedure the pups will be placed in a cage separate from the one where the mother is located
2) The foot will be sprayed with ice-cold ethanol and placed on ice to provide local hypothermic anesthesia before the clipping of the toe.
3) A sterile scalpel, razor blade or sharp scissors will be used to cleanly remove the digit.
4) If necessary, direct pressure will be used to stop any bleeding prior to returning the animal to its cage.
5) No more than two toes per foot, two feet per animal will be clipped. The tissue material obtained from toe-clipping will be used also for genotyping analysis.
6) All pups must be observed for at least 24 hours post-clipping for lethargy or failure to thrive (in rodents <10 days old, by monitoring the milk spot in their abdomen to determine if they are feeding appropriately, in rodents >10 days old, by monitoring their mentation, hydration and presence within the nest). Pups that fail to recover or are rejected by their mother must be euthanized and removed from the study.

1.8 Image or Sound Recordings of Laboratory Animals

This section describes under what conditions photographing (cameras/cell phones), video-recording and sound-recording devices are permitted in any area where animals are housed, tested or used at Stony Brook University, including, but not limited to, the Animal Care Facility (DLAR), research/teaching laboratories, field study locations, and university vehicles/vessels.

Stony Brook University is aware that images/recordings may be needed for various reasons, including research, training or documentation of clinical cases. Images must only be utilized for scholarly or scientific purposes. This section accommodates the scientific needs of those who require photographs, filming and/or recordings for collection or dissemination of their research data.

In general, the use of any photographic, video or recordings devices is prohibited, unless it is approved as part of your IACUC application. When photographing, videotaping and/or audio-recording of the animals or animal facilities is proposed for research

purposes, the IACUC proposal must include a scientific justification for the recording device(s).

The use of a cell phone/camera and other devices capable of capturing images/recordings of animals or animal facilities for personal use is strictly prohibited. Only Stony Brook IACUC approved personnel are permitted to take pictures, movies or sound recordings of research animals for the purpose of recording instructional/research activities, and this must be described in the IACUC-approved protocol.

1) The DLAR Director must be contacted whenever coordinating a visit with an external entity/outside media crew(s).
2) The PI must submit and secure an amendment approval from the IACUC documenting the reason for this camera/filming.
3) The PI must obtain permission in advance for the external entity/outside media crew to be on the premises through the Stony Brook University Media Relations Officer. The outside entity will be required to sign a Media Use Agreement.
4) Visiting faculty, visiting scientists, prospective employees, prospective students, etc. are **not** permitted to capture images or sound recordings in the DLAR or in research/teaching laboratories.
5) Those who do not follow these guidelines are subject to immediate suspension of animal work privileges and access pending IACUC review and further action.
6) Anyone found taking unauthorized photographs or recordings will be immediately asked to leave the facility and must relinquish images or other recordings obtained without permission.
7) All DLAR employees, taking unauthorized photos or recordings and failure to follow these guidelines are grounds for disciplinary action, up to and including termination.

1.8.1 Photographs/Recordings

1) All procedures depicted in photographs/recordings must be described and approved in the IACUC submission.
2) No cage cards, building names, room numbers, or employee badges/IDs can be visible.
3) All personnel visible must be wearing appropriate Personal Protective Equipment (PPE).
4) Appropriate safety, handling and restraint methods for the species must be used.
5) Images should only show the necessary parts of the animal to accurately depict the approved experiment and all surroundings (cages or pens, accessories) must be clean and tidy.
6) Animal should be appropriately draped.
7) Water bottles and feeders can be visible in the photograph.
8) If the animal is deceased, the animal should be placed on a clean drape and the area surrounding the animal must be free of any bodily fluids.

9) Images of abnormalities/ lesions should be draped, framed and/or cropped to minimize viewing of the animal.

1.8.2 Security

1) If video-monitoring or audio-monitoring remotely, a description of the duration of the recording/taping, the device(s) on which the monitored activities will display and safeguards established to ensure that non-research staff do not have access to such recordings must be provided in your IACUC application. Remote access should be conducted via VPN.
2) Recordings shall not be streamed/saved to personal hand-held devices and images and/or audio-recordings of research animals shall not be posted to any type of social media (Facebook, Instagram, etc.), news venues, or laboratory websites, unless permitted by the Vice President for Research, the DLAR Director and the Media Relations Officer.
3) Any removable storage devices (tapes, flash drives, etc.) that contain images and sound recordings of animals must be encrypted and kept in a secure, locked area with limited access with two-factor authentication, such as password-protection in addition to required encryption.
4) Computers containing images and/or audio-recordings must be password-protected and the images/recordings must be destroyed as soon as no longer required for the research. Recordings should be promptly downloaded to a secured drive and not stored for longer than necessary on the camera, video recording device, an unsecured computer hard drive or other unsecured external storage device.

1.8.3 Exemptions

1) When performed by or at the direction of the DLAR veterinary staff for the purpose of diagnosing or documenting clinical disease, veterinary care, or treatment.
2) When performed by IACUC members or office staff to document conditions during IACUC mandated inspections.
3) When performed by government inspectors (e.g. USDA Veterinary Medical Officer) when required to document condition of facility, compliance or animal welfare issues.
4) If an investigator is personally taking photographs/video for the purpose of publication or conferences, to include in a grant application, or to share with a project collaborator, he/she does not need to request approval for these activities in an IACUC protocol.
5) If an investigator is taking photographs/video for the purpose of getting IACUC/DLAR feedback related to an adverse effect.

1.9 Calorimetry Measurements in Rats and Mice

1.9.1 Metabolic Rate Measurements

Indirect calorimetry is a non-invasive method to study in vivo metabolic substrate utilization and basal metabolic rate of rodents. The calorimeter is made by Oxymax and is an open circuit, indirect calorimeter system, meaning that air is pumped through 8 test chambers and the gas sensors and then expelled into the atmosphere. The percent O2 and CO2 gas levels of each test chamber environment are measured periodically and the changes in the levels are used to compute the O2 consumption (VO2) and CO2 production (VCO2) of individual animals.

1.9.2 Activity Level Measurements

The Oxymax is also equipped with four Opto-M3 Activity Meter pairs on each test chamber. Each pair consists of an Emitter, which sends out pulsed infrared beams, and a Detector, which picks up these optical pulses. When a beam is broken by the animal's movement, a Beam Breaking Count is recorded and subsequently accumulated. By recording the accumulated counts, it is possible to compare amounts of movements of individual animals.

1.9.3 Body Temperature Measurements

Animal's body temperature can be measured using Oaklon IR Temp Gun (Oaklon, Vernon Hills, IL) and Digi-Sense Thermocouple Thermometer (Cole-Parmer, Vernon Hills, IL), in order to correlate metabolic function with energy expenditure. Oaklon IR Temp Gun is a non-invasive device that uses infrared technology measuring surface temperature of the animals. Alternatively, the temperature may be obtained using a Digi-Sense Thermocouple Thermometer that has a thin (0.05 inch diameter) and a smooth end probe that is inserted approximately 4 mm into the anus of the animal, allowing measurement of animal's core body temperature. Animals are manually restrained when the thermocouple method is used.

1.9.4 Experimental Procedures

1) An individual animal is housed in a single test chamber (size, 8" x 5" x 4"). Maximum 8 animals can be used for each experiment (the Oxymax system has 8 test chambers). Animals have free access to food and water during the entire experiment period.
2) During a typical experiment, individual mice/rat will be housed in a test chamber for 3-7 days (for short term studies) or up to 1 month**. NOTE:** THIS IS CONSIDERED AN EXCEPTION TO THE GUIDE AND SHOULD BE MADE VERY CLEAR IN A COVER LETTER. Be sure to upload the Long-Term Animal Housing Form; for long term studies and if housed outside of an approved DLAR facility. The Oxymax system will continuously measure and compute VO2, VCO2, RER, Heat, and Activity during the entire experiment period. The body temperature may be taken periodically throughout the length of the study.

3) The test chambers are cleaned twice a week, first with detergents and then with 5% bleach solution, as recommended by the manufacturer.

1.9.4.1 Adverse effects

The procedures listed above, with the exception of the use of the temperature probe, are non-invasive and do not require any manipulation of the animal.

1.9.4.2 Clinical Monitoring and Management

Animals are monitored at least twice daily while they are in the test chambers. Any animal that does not appear normal in any way will be reported to the clinical veterinarian and any recommendations for treatment or euthanasia will be followed.

1.10 Surgical Collection of Oocytes from Frogs

1.10.1 Anesthesia (required for surgical removal of oocytes)

1) Frogs are anesthetized by immersion in a Tricaine (Tricaine methanesulphonate) solution. Tricaine should be adjusted to neutral pH.
   - Tadpoles/larvae – 0.05% solution (0.5 g/L)
   - Adults frogs/urodeles - 0.1-0.2% solution (1-2g/L)
   - Toads (Bufo sp.) – 0.3% solution (3g/L)
2) Anesthesia is maintained by keeping the frog partially immersed in a small amount of Tricaine solution during the surgical procedure.
3) Ice should <u>not</u> be added to the anesthetic solution.
4) The use of any other anesthetic agents must be justified in the IACUC application.

1.10.2 Anesthesia Monitoring

1) A light plane of anesthesia is characterized by a loss of righting reflex and corneal reflex. The withdrawal reflex, spontaneous movement and cardiac impulse (visible heartbeat) are retained.
2) A deep plane of anesthesia is the stage when only the cardiac impulse is present. The withdrawal reflex is the last to go.
3) While inducing, and during the procedure, the following parameters must be monitored at a minimum of 5-minute intervals:
   - Respiratory rate
   - Righting and corneal reflex
   - Response to noxious stimulus
   - Spontaneous movement
   - Cardiac impulse (visible beating)

4) A surgical plane of anesthesia should occur within 20 minutes of immersion into the solution. Erythema of the ventrum or other light skinned areas of the body is the first sign of anesthesia induction.

1.10.3 Anesthesia Recovery Monitoring

1) Rinsing the body in distilled, well-oxygenated water will reverse the anesthetic effects.
2) During recovery from anesthesia, the following clinical parameters must be monitored at a minimum of 5-minute intervals until the animal is ambulatory.
   - Respiratory rate
   - Spontaneous movement
   - Muscle tone
3) Recovery should take approximately 15 minutes.

1.10.4 Surgical Procedure

1) After the animal attains a surgical plane of anesthesia (confirm with toe pinch) a gauze pad, soaked in betadine solution, should be applied to the abdomen. Do not rub the skin with betadine.
2) Surgery should be performed using aseptic techniques:
   - The surgeon should wear mask, sterile gloves, a lab coat and a head cover.
   - All instruments must be pre- sterilized by acceptable methods, including steam sterilization, Cidex™ cold sterilization or by the use of a glass bead sterilizer. Instruments must be re-sterilized between animals. When performing surgery on more than one animal, effective sterilization can only be practically achieved by use of a glass bead sterilizer or by pre-sterilization of multiple sets of instruments. Cidex™ cold sterilization requires 10 hours of contact time to be effective. Dipping instruments in 70% alcohol between surgeries does not achieve sterility (>30 hrs of contact time required) and is not an acceptable method.
3) A 1-2 cm. incision is made in the abdomen, parallel and lateral to the midline.
4) A section of ovary containing several dozen oocytes is removed. Avoid exteriorizing the ovary outside of the abdominal cavity.
5) Place one absorbable suture in the ovary to control bleeding.
6) Suture the abdominal muscle and skin.
7) The surgical procedure takes approximately 15 minutes.

Note: No analgesia is required for this procedure

1.10.5 Surgical Parameters

1) If frogs are to have multiple survival surgeries performed, tissue should be removed from the other ovary and surgery should not be performed until the initial incision has completely healed (approximately 2-3 months).
2) The number of multiple survival surgeries performed on any one animal should be determined by the animal's clinical appearance (i.e. incisions that have healed properly, maintenance of weight, good activity level) and quality of oocytes produced.
3) No more than 5 multiple survival surgeries may be performed on any one animal. Additional multiple survival surgeries must be justified by the investigator and approved by the IACUC.

1.10.6 Adverse Effects

Potential adverse effects from these surgical procedures include:

- Anesthetic related respiratory distress
- infection of the surgical site
- Non-healing of the surgical site
- Peritonitis

1.10.7 Clinical Monitoring and Management

1) Frogs must be segregated post-operatively into a small recovery tank overnight. Then, when returned to the animal facility, the frogs are placed in a separate tank with the surgical date clearly indicated on the tank, for a minimum of 2 weeks to facilitate monitoring.
2) Frogs must be monitored at least twice weekly after each surgical procedure.
3) Any frog with an incision that is not healing properly (open, infected, red) or animals that appear to be losing weight should be reported to the DLAR Veterinary staff so that appropriate medical treatment can be initiated.
4) If animals have acute adverse reactions to the anesthetic agents (respiratory distress and/or lack of recovery), they must be euthanized immediately unless the approved IACUC protocol has approve additional uses.

1.10.8 Early Endpoints

1) Animals should be euthanized if the surgical incision dehisces or if treatment of an infected incision site is unsuccessful.
2) After an animal has had 5 multiple survival surgeries performed (as evidenced by the number of surgical scars on the abdomen), the animal should be euthanized.

1.11 Use of Avian Embryos

Avian embryos are not considered live animals by U.S. regulatory agencies and many universities do not regulate their use in research. Nonetheless, there is a consensus in

the scientific community that avian embryos greater than two thirds of the way to hatching can experience pain. If avian embryos hatch, intentionally or unintentionally, they are live vertebrate animals and are regulated by the IACUC. Consequently, the IACUC has adopted the following guidelines.

Chick embryos are considered the model species. If other avian species are used, then the guidelines should be adjusted based on relative time to hatching.

1) Research involving avian embryos that will be euthanized prior to 3 days before hatching does not require IACUC review, as these are not considered to be live vertebrate animals. The IACUC does require submission of a complete animal protocol for projects utilizing pre-hatched avian embryos at or after 80% of the mean incubation period.
2) Chick embryos younger than embryonic day 15 (E15) are assumed to be unable to experience pain. It is recommended that E14 or younger embryos be euthanized by hypothermia, typically by placing the eggs in a -20°C freezer.
3) Chick embryos from E15 to E17 can experience pain and should be euthanized by decapitation or other rapid and humane method.
4) Embryos E18 and older must be euthanized by humane methods such as C02, anesthetic agents or decapitation. It should be noted that embryos are resistant to C02. If this method is chosen, the embryos must be exposed to 100% C02 for at least 20 min. Dry ice is unacceptable as a source of C02 for euthanasia.
5) The IACUC recognizes that inadvertent hatching may occur. Investigators are asked to describe their methods for humane euthanasia of hatchlings.
6) These guidelines are based on recommendations of ILAR, the NIH intramural recommendations for rodent neonates, and the AVMA Panel on Euthanasia.

1.12 Rodent Survival Surgery

This section applies to all surgical procedures performed on rodents in which the animals are expected to recover from anesthesia. Prior to performing any survival surgery techniques on rodents, an approved IACUC protocol must be in place with appropriately trained personnel and procedures available.

The following must be adhered to:

1) Appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices are required.
2) A dedicated surgical facility is not required.
3) All survival surgery will be performed by using aseptic procedures, including masks, sterile gloves, sterile instruments, and aseptic techniques

It is important for research personnel to be appropriately qualified and trained in all procedures to ensure that good surgical technique is practiced.
Good technique includes:

- Asepsis,

- Gentle tissue handling,
- Minimal dissection of tissue,
- Appropriate use of instruments,
- Effective hemostasis, and
- Correct use of suture materials and patterns.

1.12.1 Intra-operative and Anesthesia Records

Animals under anesthesia be carefully monitored to insure adequate depth of anesthesia, animal homeostasis, timely attention to problems, and support during anesthetic recovery. Monitoring includes, but is not limited to, checking anesthetic depth and physiological parameters (minimum: heart rate and respiratory rate) on a regular basis (minimum every 15 minutes).

Record keeping is an essential component of peri-operative care. For all surgical procedures, an intra-operative anesthetic record must be kept and included with the surgeon's report as part of the animal's records. In addition to the above requirements, the record should include all drugs administered to the animal, noting the dose, time, and route of administration. These records should be available to the IACUC and DLAR staff and any other personnel providing post-operative care.

The required monitoring will vary according to the species and the complexity of the procedure, but should include:

- Adequate monitoring of anesthetic depth and homeostasis
- Support such as fluid supplementation, external heat, or ventilation
- Monitoring and support during anesthetic recovery
- Post-operative monitoring

The following are required for monitoring anesthetized animals

- Circulation: to ensure that blood flow to the tissues is adequate.
  Methods: Heart rate, palpation of peripheral pulses, ECG, auscultation of heartbeat, non-invasive or invasive blood pressure monitoring.
- Oxygenation: to ensure adequate oxygen concentration in the animal's arterial blood.
  Methods: observation of mucous membranes color and CRT (should be less than 2 seconds if circulation is adequate), pulse oximetry, blood gas analysis
- Ventilation: to ensure that the animal's ventilation is adequately maintained.
  Methods: respiratory rate, observation of thoracic wall movement or breathing bag movement if animal is spontaneously breathing, auscultation of breath sounds, respiratory monitor, capnography, blood gas monitoring.

The records should include notations for all monitoring criteria indicated in the IACUC protocol.

Aseptic Procedures / Personal Protective Equipment

- Clean lab coat
- Mask
- Sterile surgical gloves
- Head cover
- Additional equipment as listed on the approved protocol.

Pre-Operative

Note: If weight monitoring or other observations are a part of your protocol, be sure to strictly adhere to the schedule approved in your application and maintain records of all parameters. Get a pre-procedure weight.

1) Surgery should be conducted in a disinfected, uncluttered area that promotes asepsis during surgery (see Appendix, Table 1). Use new underpads, towels. Replace these materials after each surgery session.
2) The instruments, sutures, etc. should be placed in a specially designed pack or wrapped in drapes or cloths, then steam autoclaved. Label autoclaved packs with date of sterilization. Any implants should be sterilized; fragile implants may be gas-sterilized or soaked in a 2% glutaraldehyde solution (soak for 10 hours) or other chemical sterilant (not disinfectant) - rinse off with sterile water before implanting.
3) Preparation of the Animal
    - The fur must be removed from the surgical site, either by clipping, plucking or using a depilatory.
    - An area approximately 15% larger than the area of the incision should be prepared.
    - All loose fur should be vacuumed away, or sticky tape may be used.
    - Clean and aseptically prepare the surgical site by using an appropriate scrubbing technique (e.g. scrubbing in gradually enlarging circular pattern from the interior of the shaved area to the exterior) and an effective disinfectant - e.g. alternating Betadine or Nolvasan™ and alcohol scrubs X 3. (See Appendix, Table 2). Perform this procedure in an area separate from where the surgery is to be conducted.
4) The surgeon must wear a clean lab coat, mask and sterile gloves. A sterile surgical gown and head cover are recommended for major or prolonged surgeries. Surgeons should wash and dry their hands before aseptically donning sterile surgical gloves.

Operative

1) The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
2) Begin surgery with sterile instruments and handle instruments aseptically (see Appendix, Table 3).
3) When using “tips-only” technique, the sterility of the instrument tips must be maintained throughout the procedure.

4) Instruments and gloves may be used for a series of similar surgeries provided they are maintained clean and disinfected between animals (see Appendix, Table 4).
5) Monitor and/or maintain the animal's vital signs.
6) Close surgical wounds using appropriate techniques and materials (see Appendix, Table 5).

Tips on Maintaining Asepsis

1) Gloved hands should be held elevated above the waist and should touch only the surgical incision and sterile objects, i.e. sterile instrument tray, sterile drape.
2) Once gloved, do not touch or lean over a non-sterile area. Do not drop your hands to your sides. Do not touch gloves to your skin or clothes. Gloves must be changed if they come in contact with a non-sterile surface.
3) Always lift an instrument from a sterile pouch or sterile surface. Do not drag instruments over the pack/drape edges because they can become contaminated. Use a sterilized area (surgical tray or sterile gauze) to rest materials on when not in use.
4) Do not allow surgical instruments to fall below the edge of the table. If an instrument does fall, the instrument is considered unsterile and should not be picked up and reused until re-sterilized. Two separate sets of surgical instruments can be used – one set for incising and manipulating skin and another for manipulating deeper tissues.
5) Sterile surfaces are to be kept dry. Moisture can lead to contamination of the surgical area. Manipulation of tissues within the surgical field with gloved hands should be avoided; the ends of sterilized instruments should be used to manipulate and handle tissues. The exteriorizing of organs should be avoided if possible, but if required, they should be placed on the sterile drape.

Multiple Surgeries in a Single Session

1) Animal procedures, materials and area preparation must be completed prior to donning sterile gloves.
2) Instruments and gloves may be used for a series of similar surgeries provided they are appropriately cleaned and disinfected between use (see Table 5 below). Note: alcohol alone is not an acceptable disinfectant.
3) If using a glass bead sterilizer, recognize that only the tips of the instruments are sterile and tips must be allowed to cool before touching tissue.
4) Animals will be monitored every 10-15 minutes, unless protocol specified more frequently, by the surgeon.
5) Alternating 2 sets of sterile instruments is a method to provide the necessary time for instruments to sit in disinfectant for the required time. Instruments must be thoroughly rinsed with sterile saline prior to re-use.
6) For major surgeries it is highly recommended that sterile gloves be changed between animals.

Post-Operative

1) Move the animal to a warm, dry area and monitor it during recovery. Return the animal to its routine housing only after it has fully recovered from anesthesia.
2) Provide analgesics as appropriate and approved in your IACUC protocol.
3) Generally, remove skin closures 10 to 14 days post-operatively.
4) Maintain a surgical record as described above.
5) Check animals at least daily for the first 3 days after surgery and make daily notations in the record about the animal's disposition and if additional analgesia or other treatment is provided.
6) If weight monitoring or other observations are a part of your protocol, be sure to

Table 1. Recommended Hard Surface Disinfectants (e.g., table tops, equipment)
Always follow manufacturer's instructions for dilution and expiration periods.

AGENT	EXAMPLES *	COMMENTS
Alcohols	70% ethyl alcohol 85% isopropyl alcohol	Alcohol is not recommended as it is a poor disinfectant with very long contact time required. Not effective on adenovirus.
Quaternary Ammonium	Roccal®, Quatricide®	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox ® 10% solution) Chlorine dioxide (Clidox®, Alcide®, MB-10®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact.
Glutaraldehydes	Glutaraldehydes (Cidex®, Cetylcide®, Cide Wipes®)	Rapidly disinfects surfaces.
Phenolics	Lysol®, TBQ®	Less affected by organic material than other disinfectants.
Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.

* The use of common brand names as examples does not indicate a product endorsement.

Table 2. Skin Disinfectants
Alternating disinfectants is more effective than using a single agent. For example, an iodophor scrub can be alternated three times with 70% alcohol, followed by a final soaking with a disinfectant solution. Alcohol, by itself, is not an adequate skin disinfectant. The evaporation of alcohol can induce hypothermia in small animals.

Agent	Examples *	Comments
Iodophors	Betadine®, Prepodyne®, Wescodyne®	Reduced activity in presence of organic matter. Wide range of microbicidal action. Works best in pH 6-7.
Cholorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.

* The use of common brand names as examples does not indicate a product endorsement.

Table 3. Recommended Instrument Sterilants
Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

Agent	Examples *	Comments
Steam sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (e.g., 121oC for 15 min. vs 131oC for 3 min).
Dry Heat	Hot Bead Sterilizer Dry Chamber	Fast. Instruments must be cooled before contacting tissue. *Only tips of instruments are sterilized with hot beads.*
Gas sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue, a suspected human carcinogen and toxic to the reproductive system. All materials require safe airing time.
Chlorine	Chlorine Dioxide	Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Glutaraldehydes	Glutaraldehyde (Cidex®, Cetylcide®, Metricide®)	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.
Hydrogen peroxide-acetic acid	Actril®, Spor-Klenz®	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.

* The use of common brand names as examples does not indicate a product endorsement.

Table 4. Recommended Instrument Disinfectants
Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

Agent	Examples *	Comments
Chlorine	Sodium hypochlorite (Clorox ® 10% solution) Chlorine dioxide (Clidox®, Alcide®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh. Kills vegetative organisms within 3 min. Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Instruments must be rinsed with sterile saline or sterile water before use.

* The use of common brand names as examples does not indicate a product endorsement.

Table 5. Wound Closure Selection

Material*	Characteristics and Frequent Uses
Polyglactin 910 (Vicryl®), Polyglycolic acid (Dexon®)	Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.
Polydiaxanone (PDS®) or, Polyglyconate (Maxon®)	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable
Polypropylene (Prolene®)	Nonabsorbable. Inert.
Nylon (Ethilon®)	Nonabsorbable. Inert. General closure.
Silk	Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). Excellent handling. Preferred for cardiovascular procedures.
Chromic Gut	Absorbable. Versatile material.
Stainless Steel Wound Clips, Staples	Nonabsorbable. Requires instrument for removal.
Cyanoacrylate (Vetbond®, Nexaband®)	Skin glue. For non-tension bearing wounds.

* The use of common brand names as examples does not indicate a product endorsement.

- Suture gauge selection: Use the smallest gauge suture material that will perform adequately.
- Cutting and reverse cutting needles: Provide edges that will cut through dense, difficult to penetrate tissue, such as skin.
- Non-cutting, taper point or round needles: Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as peritoneum or intestine.

1.13 Gonadectomy in Rats and Mice

1.13.1 Anesthesia

1) Animals must be anesthetized with one of the following agents for these procedures:

Mice → Ketamine (90-120 mg/kg) and Xylazine (5-10 mg/kg), IP
OR
Isoflurane (vaporizer [1-5%] or open drop to effect)

Rats → Ketamine (60-75 mg/kg) and Xylazine 5-10 mg/kg), IP
OR
Isoflurane (vaporizer [1-5%] or open drop to effect)

2) The use of any other anesthetic agents must be identified in the IACUC application.

1.13.2 Anesthesia Monitoring

During the surgical procedure the following parameters must be monitored at 5-minute intervals:

- Respiratory rate
- Response to noxious stimulus (ie. toe pinch)
- Spontaneous movement

1.13.3 Anesthesia Recovery Monitoring

1) To protect the animal from hypothermia they should never be placed on metal surfaces – place animals on a water re-circulating heating blanket or wrap them in a towel (while still allowing visible monitoring) to conserve body temperature. Thermal packs can also be used.
2) During recovery from anesthesia, the following clinical parameters must be monitored at a minimum of 15-minute intervals until the animal is ambulatory.
   - Respiratory rate
   - Movement
   - Ability to maintain sternal recumbency
3) Animals may crawl into the corners of the recovery cage and bedding can sometimes block the airway. If recovering animals are housed with cage mates, the cage mates will sometimes injure animals not responding to stimulation. Personnel should be alert to these possible complications during the recovery period.
4) It is estimated that animals will recover within 30-60 minutes postoperatively.

1.13.4 Ovariectomy

1) Weigh and anesthetize the animal.

2) When animal is properly anesthetized (reduced respiratory rate, lack of spontaneous movement to toe pinch), shave both sides of the flank. In the mouse, fur may be plucked. Swab the shaved area with 70% alcohol & betadine/chlorhexidine.
3) Aseptic surgical techniques must be used for this procedure:
   - Personnel must wear a lab coat (or surgical gown), mask, head cover, and sterile gloves.
   - All instruments must be cleaned and pre-sterilized by acceptable methods, including steam sterilization, Cidex™ cold sterilization or by the use of a glass bead sterilizer. Instruments must be re-sterilized between animals. When performing surgery on more than one animal, effective sterilization can best be achieved by using either a glass bead sterilizer or by pre-sterilization of multiple sets of instruments. Cidex™ cold sterilization requires 10 hours of contact time to be effective. Dipping instruments in

70% alcohol between surgeries does not achieve sterility (>30 hrs of contact time required) and is not an acceptable method.
    - The surgical site must be covered with a sterile drape or sterile, clear surgical adhesive material. The size of the drape should be adjusted to the size of the animal so that aseptic techniques can be maintained and the animal properly monitored.
4) A 5 mm, dorsal incision is made through the skin. The incision is centered between the dorsal hump and the base of the tail. In the rat, a 10 mm incision is placed in a similar fashion. The skin is separated from the underlying muscle.
5) Locate the ovarian fat pad which is visible under the muscle. Rather than cutting the muscle, insert the tip of a double blunt/blunt iridectomy or tissue scissors just through the muscle layer, and separate the muscle fibers by opening the scissors in a dorsal/ventral direction.
6) Hold the edge of the incision open with a small rat toothed forceps and pull the fat pad through the incision with a blunt forceps. The ovary will be attached to the fat pad.
7) A mosquito forceps is placed at the boundary between the oviduct and uterus and a ligature placed just below the hemostat. After removing the ovary and oviduct with scissors, release the hemostat and make sure no bleeding occurs. Return the uterine horn to the abdominal cavity. Suture the abdominal wall with 2 absorbable sutures. The skin should be closed with skin clips, non-absorbable suture material or skin glue. Skin clips and non-absorbable suture material should be removed 7-14 days post-operatively.
8) Repeat 4-7 on other side.
9) Return the animal to its cage and monitor recovery from anesthesia as described below.

1.13.4 Orchidectomy

1) Weigh and anesthetize the animal.
2) Once the animal is properly anesthetized (reduced respiratory rate, lack of spontaneous movement to toe pinch), shave the lower abdomen (for abdominal approach) or scrotum (for scrotal approach). In the mouse, fur may be plucked. Swab the shaved area with 70% alcohol & betadine/chlorhexidine.
3) Aseptic surgical techniques must be used for this procedure:
    - Personnel must wear a lab coat (or surgical gown), mask, head cover, and sterile gloves.
    - All instruments must be pre-sterilized by acceptable methods, including steam sterilization, Cidex™ cold sterilization or by the use of a glass bead sterilizer.
    - Instruments must be re-sterilized between animals. When performing surgery on more than one animal, effective sterilization can best be achieved by using either a glass bead sterilizer or by pre-sterilization of multiple sets of instruments.
    - Cidex™ cold sterilization requires 10 hours of contact time to be effective.
    - Dipping instruments in 70% alcohol between surgeries does not achieve sterility (>30 hrs of contact time required) and is not an acceptable method.

- The surgical site should be covered with a sterile drape or sterile, clear surgical adhesive material. The size of the drape should be adjusted to the size of the animal so that aseptic techniques can be maintained and the animal properly monitored.

1) A 1.0 cm median incision should be made through the skin at the tip of the scrotum (scrotal approach) or along the midline lower abdomen, above the prepuce (abdominal approach). Open the cremaster muscles with a 7 mm incision (scrotal approach) or the midline abdominal wall (abdominal approach).
2) Both testes can be reached through the same incision. Localize the testicular fat pad on the left side and pull it through the incision using a blunt forceps. The cauda epididymidis is pulled out together with the testis, followed by the caput epididymidis, the vas deferens and the testicular blood vessels. Place a single ligature around the vas deferens and the blood vessels. Remove the testis. Repeat for the other testis
3) After both testes have been removed, replace the remaining pieces of the vas deferens, the fat and the blood vessels back in the scrotal sac or abdomen.
4) Close the muscle layer using two resorbable 5-0 sutures and the skin with non-resorbable 4-0 suture material or skin clips. Skin clips and non-absorbable suture material should be removed 7-14 days post-operatively. (note: scrotal skin clips can be used to close a scrotal incision, but they can affect sexual proclivity)
5) Return the animal to its cage and monitor anesthesia recovery as described below.

1.13.6 Analgesia

At least a single dose of analgesics will be administered post-operatively (Buprenorphine 0.01-0.05 mg/kg and/or Ketorolac 4 mg/kg SC). The animal should be evaluated at least daily for 3 days and additional analgesics given as needed. Local analgesics (Marcaine, Bupivacaine) may also be used at the incision site.

1.13.7 Adverse Effects

1) Potential adverse effects from these surgical procedures include:
   - Anesthetic related respiratory distress
   - Postoperative infection
   - Wound dehiscence
   - Postoperative pain as evidenced by:
     - Decreased activity (lethargy)
     - Decreased food and water intake
     - Rough hair coat, hunched posture
     - Weight loss (no greater than 15% permitted), hunched posture
     - Vocalization when the incision is touched
     - Isolation of the individual from the group
2) Adverse effects can be prevented or limited using aseptic surgical techniques and proper choice of suture materials, as described above.

1.13.7 Clinical Monitoring and Management

Postoperatively animals must be monitored once daily for 3-5 days, including weekends and holidays by the investigator staff. Notations must be made into the required surgical record. If no adverse effects are seen, the animals should then be monitored once to twice weekly.

1) "Rodent Post-operative Monitoring Sheets" can be used to assist investigators with pain assessment.
2) If signs of post-operative pain (see Section D), wound dehiscence, or postoperative infections are seen, consult immediately with the DLAR veterinary staff regarding treatment options. If animals have acute adverse reactions to the anesthetic agents (respiratory distress and/or lack of recovery) or acute wound dehiscence, they must be euthanized immediately.

1.13.8 Early Endpoints

If animals are experiencing weight loss (15% greater than initial body weight), have wound infections that are non-responsive to therapeutic intervention, or have major surgical dehiscence, they should be immediately euthanized.

1.14 Implantation of Drug Delivery Systems in Rodents

1.14.1 Anesthesia

1.14.1.1Pre-emptive Analgesia

1) Administration of Buprenorphine delivered SQ 15-60 min before induction of anesthesia is recommended. Alternatively, post-operative analgesia may be used (see section on Post-operative Analgesia below).
2) The long duration of action (4-12 hours) of this agent allows it to (a) act as a pre-anesthetic to facilitate further handling and decrease anxiety/stress and (b) alleviate any potential pain during the immediate post-operative period.
3) Analgesic dosing is as follows: Rats – 0.1-0.5 mg/kg, SQ; Mice – 0.05-0.1 mg/kg, SQ

1.14.1.2 Anesthetic Protocols

1) Anesthesia is required for surgical implantation of the drug delivery system
2) Anesthetic Agents – animals will be anesthetized using one of the three following agents or combination of agents:

   Rats – Ketamine/Xylazine – 60-75 mg/kg / 5-10 mg/kg, IP
   Isoflurane via vaporizer 1-5% or open drop method

Mice – Ketamine/Xylazine – 90-120 mg/kg / 5-10 mg/kg, IP
Isoflurane via vaporizer 1-5% or open drop method

3) Use of any other anesthetic agents must be identified in the IACUC application.

1.14.1.3 Anesthesia Monitoring

Prior to and during the procedure, the following parameters will be monitored at ~5 min intervals:

- Respiratory rate
- Response to noxious stimulus (*e.g.*, withdrawal in response to toe pinch)
- Spontaneous movement

1.14.1.4 Anesthesia Recovery Monitoring

1) It is estimated that animals will require at least 15 minutes (isoflurane) or 60 minutes (injectable anesthetics) to fully recover from anesthesia. During this recovery period, the following parameters must be monitored at 15 min intervals until the animal is ambulatory.
   - Respiratory rate
   - Movement
   - Ability to maintain sternal recumbency
2) During recovery from anesthesia, the animal should be placed on a pre-warmed circulating water heating pad, a heating blanket, or covered well to maintain body temperature. Animals should never be placed on a metal surface.

1.14.2 Surgical Procedures

Note: All surgical procedures must follow the Guidelines for Rodent Survival Surgery at Stony Brook University.

1.14.2.1 Subcutaneous (SC) Implantation

1) SC implantation is the easiest and least invasive procedure.
2) The most common site for SC implantation in rats and mice is on the back between and slightly posterior to the scapulae. Other regions may be used, provided that the pump does not put pressure on the vital organs or impede respiration.
3) To implant the pump SC -

Step 1 – Shave and disinfect the back.
Step 2 – Make a mid-scapular incision in the skin.

Step 3 – Using blunt dissection, make a subcutaneous pocket for the pump. The pocket should be large enough to allow some free movement of the pump, but not so large that the pump can slip down on the flank of the animal.
Step 4 - Insert the filled pump into the pocket, delivery port first. Be sure that the pump does not rest directly beneath the incision, as this could interfere with wound healing.
Step 5 – Close the incision with wound clips or sutures and seal the incision with sterile tissue adhesive to help prevent infection. Wound clips and non-absorbable suture materials should be removed 7-14 days post-operatively.
Step 6 - Administer the appropriate post-operative analgesic and monitor the animal for return of normal behaviors.

1.14.2.2 Intraperitoneal (IP) Implantation

Note: The IP route should probably be avoided with agents that have a significant first-pass effect because a majority of the dose of any substance administered IP may be absorbed via the hepatic portal circulation rather than by the capillaries.

1) IP implantation is appropriate in animals with sufficiently large peritoneal cavities. Depending on the size of the animal relative to the pump, however, IP implantation can disrupt normal feeding and weight gain for 1-2 days
2) Allow 24-48 hours for the animal to recover after IP implantation.
3) To implant the pump IP -

Step 1 – Shave and disinfect the skin over the abdomen.
Step 2 – Make a midline skin incision, slightly larger than the diameter of the pump, in the lower abdomen just below the ribcage.
Step 3 – Carefully tent-up the musculoperitoneal layer to avoid damage to the bowel and incise the peritoneal wall directly beneath the cutaneous incision.
Step 4 – Insert the filled pump, delivery port first, into the peritoneal cavity.
Step 5 – Close the musculoperitoneal layer with 4.0 absorbable sutures in an interrupted or continuous pattern, taking care to avoid perforation of the underlying bowel.
Step 6 – Close the skin incision with wound clips or sutures and seal the incision with sterile tissue adhesive to help prevent infection. Wound clips and non-absorbable suture materials should be removed 7-14 days postoperatively.
Step 7 - Administer the appropriate post-operative analgesic and monitor the animal for return of normal behaviors.

1.14.2.3 Adverse Effects

1) Potential adverse effects from this procedure are minimal, but may include the following:
   - Anesthetic-related respiratory depression
   - Infection of the subcutaneous pocket or abdominal cavity
   - Post-operative pain as evidenced by:

- Decreased activity
- Decreased food and water intake
- Weight loss
- Vocalizations
- Rough hair coat
- Hunched posture

2) Adverse anesthetic effects can be minimized by proper dosing of anesthetic agents and careful monitoring of animals during the anesthetic period.
3) Infection can be prevented or minimized if trained surgeons use aseptic surgical techniques and maintain the sterility of products being used. Administration of prophylactic antibiotics may be useful in minimizing the risk of infection, and this should be discussed with the Clinical Veterinarian.

1.14.3 Post-operative Care

1.14.3.1 Post-operative Analgesia

Analgesia will be provided under the direction of the Clinical Veterinarian, but will include at least a single post-operative dose of one of the following:

Rats – Buprenorphine – 0.1-0.5 mg/kg, SQ (lasts 8-12 hours)
Ketorolac – 4 mg/kg, SQ (lasts 24 hours)

Mice – Buprenorphine – 0.05-0.1 mg/kg, SQ (lasts 8-12 hours)
Ketorolac – 4 mg/kg, SQ

Note: Additional doses will be administered, as needed until the animal is not showing any signs of pain (see above list).

1.14.4 Clinical Monitoring and Management

1) Animals should be monitored daily until the wound clips or sutures are removed (7-14 days), and then once to twice weekly until completion of the study.
2) To assist investigators with pain assessment, the "Rodent Post-operative Monitoring sheet" can be used.
3) If any adverse effects are seen, the Clinical veterinarian will be informed immediately and appropriate treatment will be administered. Animals not responding to treatment will be euthanized.

1.14.5 Early End Points

1) Animals that exhibit an acute adverse reaction to the anesthetic agent (*e.g.*, respiratory distress) or weight loss > 15% will be euthanized immediately.

2) Animals that show any signs of pain/distress (as outlined above) or swelling/discharge from the incision site will be removed from the study and reported to the Clinical Veterinarian. If deemed untreatable, these animals will be euthanized.

Note: Methods for the standard protocol for implantation of drug delivery systems in rodents – all procedures described are according to the recommendations by Alzet Technical Information Manual and Alzet surgical implantation video clips.

1.15 Preclinical MRI of Rats/Mice

1.15.1 Anesthesia

1) Anesthesia is required for MRI imaging. Animals will be under anesthesia no more than 2 hours for mice and 4 hours for rats.
2) Anesthetic Agents – animals will be anesthetized with one of the following agent(s) for this procedure:

   Mice - Isoflurane 1-2.5%
   Ketamine (90 mg/kg) and Xylazine (10 mg/kg), IP
   Pentobarbital (50mg/kg), IP
   Brevital (25-50mg/kg IP) followed by Isoflurane (1-2.5%)

   Rats - Isoflurane 1-2.5%
   Ketamine (75 mg/kg) and Xylazine 10 mg/kg), IP
   Pentobarbital (50 mg/kg), IP
   Brevital (25-50mg/kg IP) followed by Isoflurane (1-2.5%)

3) Use of any other anesthetic agents must be identified in the associated IACUC application.
4) Additional agents that may be administered include:
   - Lactated Ringers solution (4 cc/kg/hr IP) or saline to maintain hydration in studies longer than 2 hours
   - Eye lubricant and cotton/gauze ear plugs
   - Various contrast agents clearly described in the associated IACUC protocol.

1.15.2 Anesthesia Monitoring

Please list the length of the scanning procedure and describe the anesthetic monitoring (if any) that will be done in Section H2 of the protocol.

- Rectal temperature
- Respiration rate
- Heart rate
- Blood pressure and/or blood gas sampling (optional)

Rats may be instrument for invasive arterial blood pressure monitoring and blood gas sampling. For this procedure, rats will be anesthetized with 2% isoflurane or alternative anesthetics.

- A femoral artery and a femoral vein will be exposed via cutdown on the medial surface of the hindlimbs using aseptic technique.
- Each blood vessel will be ligated distally with a single interrupted suture and then cannulated proximally using PE tubing of appropriate size per species.
- Each cannula will be tunneled subcutaneously to exit through the skin on the dorsum.
- The incisions will be closed with suture or wound clips.

1.15.3 Imaging the animal

- After anesthetic induction, the animal will be placed in an acrylic holder, instrumented with monitoring equipment, and imaged.
- Mouse studies lasting longer than 4 hours will be non-survival with the animal euthanized before anesthetic recovery. If time is greater than four hours, this must be justified in the application.
- The specific imaging schedule, including total number of imaging sessions and length of each session, must be described in the associated IACUC protocol.
- Any contrast agents or deviations from the above procedures that will be conducted must be described in the associated protocol.

1.15.4 Adverse Effects

1) Potential adverse effects for the procedures include:
    - Anesthetic related respiratory distress
    - Peritonitis secondary to trauma IP injections.
2) Adverse anesthetic effects will be avoided by proper dosing and titration of anesthetic agents and close monitoring of animals during the anesthetic period. Only properly trained individuals will perform the injections.

1.15.5 Clinical Monitoring and Management

1) Animals on chronic studies will be monitored daily for two days following each scan.
2) If adverse effects are seen, the investigator will consult immediately with the DLAR veterinary staff regarding treatment options.

1.15.6 Early Endpoints

If animals have acute adverse reactions to the anesthetic agents (respiratory distress and/or lack of recovery), they will be euthanized immediately.

If animals show any signs of peritonitis including inappetence, hunched posture, inactivity they will be euthanized immediately following approved procedures (Pentobarb-based solution 100mg/kg overdose or deep 5% isoflurane anesthesia followed by bilateral thoracotomy).

1.16 Standard Ultrasound Imaging of Rats and Mice

1.16.1 Facility

1.16.1.1 Access to the Ultrasound Room

1) Access is obtained by activating your ID card for entry into the ultrasound room once training has been completed.
2) Only users who have been trained on the use of the equipment by either the Visualsonics representative or the Veterinary staff will be permitted to use the ultrasound machine. Training sessions can be scheduled with the Vet staff at mutually convenient times.

1.16.1.2 Ultrasound Room Equipment Use

1) All gas tanks must be secured properly in a stand, and chained to the wall. An extra oxygen tank will be available in case an empty tank needs to be changed during a scanning session. Advise the vet staff when a tank is emptied so it can be replaced promptly. Remember to turn off the oxygen tank when finished.
2) Each user is responsible for supplying their own Isoflurane & Nair which, if needed, can be purchased from DLAR. The unused Isoflurane can be drained from the machine at the end of the imaging session and returned to your bottle. See Vet staff for instructions, or to report any problems with the ultrasound or anesthetic machines. The ultrasound machine must be cleaned with a mild soap and dried after each use.
3) Animals should be returned to their rooms as soon as they are awake. Do not leave animals in the imaging room overnight. Maximum isolation animals should be returned to the post imaging room. The conventional animals can be returned to their original room.
4) Turn the machine “ON” and select the probe and the user prior to initiating the animal anesthesia.

1.16.1.3 Anesthesia

1) Mice and rats must be anesthetized with Isoflurane gas for the imaging sessions. The individual animal is first placed into the clear plastic induction box, then they are transferred to the heated scanning table. The nose of the animal is placed into the end of the Isoflurane gas supplying tube.
2) The use of any other anesthetic agent must be identified in the IACUC application.

1.16.1.4 Anesthesia Monitoring

During the scanning procedure, the following parameters must be monitored at a minimum of 5-minute intervals:

- Respiratory rate
- Response to noxious stimulus (i.e., toe pinch)
- Spontaneous movement

1.16.1.5 Ultrasound Scanning Procedure

1) The limbs of a rat or a mouse are taped to the scanning table. A small amount of a cardiac conducting gel is placed under each limb to allow for a physiologic monitoring and recording during the procedure. The rectal probe is inserted and secured in place by the tape to allow for body temperature monitoring and recording.
2) A small amount of epilating Nair cream is applied to the area to be scanned. The cream is removed in a few minutes by gentle scrubbing with the gauze squares. Nude mice and rats do not need to be depilated.
3) The ultrasound scanning gel is applied onto the area to be scanned, the probe is lowered, and the study and recording sessions are conducted.
4) The remnants of the gel must be removed at the conclusion of the scan to avoid the excessive loss of body heat loss.
5) The rectal probe is removed, and the animal is returned to the cage for anesthesia recovery monitoring.
6) The study is edited and saved either to the hard drive or portable media storage device.
7) The ultrasound machine is turned “OFF” at the end of the study.

1.16.1.6 Anesthesia Recovery Monitoring

1) To protect the animal from hypothermia they should never be placed on metal surfaces – place animals on a water re-circulating heating blanket, or wrap them in a towel (while still allowing visible monitoring) to conserve body temperature. Thermal packs can also be used. During recovery from anesthesia, the following clinical parameters must be monitored at a minimum of 5-minute intervals until the animal is ambulatory:
   - Respiratory rate
   - Movement
   - Ability to maintain sternal recumbency
2) It is estimated that animals will recover within 3-5 minutes post scanning with monitoring. Then return the animal to their home cage.

1.17. Vascular Perfusion Fixation of Rats and Mice

The goal of perfusion fixation is to use the vascular system of a deeply anesthetized animal to deliver fixatives to the tissues of interest. This is the optimal method of tissue preservation because the tissues are fixed before autolysis begins. Perfused tissues are less susceptible to

artifacts caused by handling. Techniques for fixation vary depending on the organ and the desired processing.

Note: Set up perfusion pump in fume hood. All work with fixatives must be done in a fume hood.

Anyone who uses formaldehyde solutions in perfusions is required to complete the Lab Safety Formaldehyde Course (ELS009) from EH&S. It is available through Blackboard. They also should review the Safety Data Sheets that provides further details of the hazards posed by its use prior to work. In addition:

- Storage should take place in a cool, ventilated area, with a tightly closed container.
- An eye wash should be available in the room with a safety shower accessible nearby.

1.17.1 Perfusion Protocol

1) Set up perfusion pump in fume hood (see below for hazards, safety concerns, and procedures for limiting exposure to the hazardous fumes created during the perfusion process); attach perfusion set and perfusion needle.
   a. First, run about 50 ml of normal water through the tubing to remove any residue.
   b. Then place open end of perfusion tube in beaker filled with 4% paraformaldehyde (in ice). The volume of solution should be scaled to size of animal - usually 200 ml for mice and 500 ml for rats will be sufficient for one animal.
   c. Open valve (turn on the pump) and adjust to a slow steady drip (20 ml/min), and then close valve (turn off the pump).
2) Set up surgery site with scissors, forceps and clamps.
3) The animal to be perfused is weighed and anesthetized with an I.P. injection of ketamine/xylazine. Use of other anesthetics must be listed in the protocol. Allow 10 to 15 minutes for anesthesia to occur, indicated by the loss of sensory/ reflex response, i.e. non- response to tail pinching or paw pinching. Once the animal is anesthetized, care should be taken to prevent heat loss. The use of a heating pad (on low setting) or lamp is recommended. There should be no direct contact with the animal from the heating source.
   - Place the animal on the operating table with its back down. You may use tape to hold the appendages so that the animal is securely fixed. Use pinch-response method to determine depth of anesthesia. Animal must be unresponsive before proceeding with the following steps.
   - Make incision with scalpel through abdomen along the base of the rib cage. With sharp scissors, cut through the tissue at the bottom of diaphragm to allow access to rib cage.
   - Cut through ribs just left of the rib cage midline.
   - Make one center or two end horizontal cuts through the rib cage, and open up thoracic cavity. Clamp open to expose heart and provide drainage for blood and fluids.

- While holding heart steady with forceps (it should still be beating), insert needle directly into protrusion of left ventricle. **Be careful not to extend the needle too far in, as it can pierce an interior wall and compromise circulation of solutions!** Secure needle position by clamping in place near the point of entry. Allow slow, steady flow of around 20 ml/min of 0.9% saline solution (or heparinized saline or PBS).
- Immediately make cut in right atrium with sharp scissors, and make sure solution is flowing freely. If fluid is not flowing freely or is coming from animal's nostrils or mouth, reposition the needle.
- When blood has been cleared from body, change to fixative (e.g., 4% paraformaldehyde) solution. 200 ml of fixative should be sufficient for a mouse; 500 ml for a rat.
- Perfusion is almost complete when spontaneous movement of the tail or paws ('formalin dance') and lightened coloration of the liver are observed. The perfusion time will vary depending on the size of the animal.
- In case of unsuccessful perfusion, cardiac puncture is the appropriate means of euthanasia.
- Fixed organs and tissues can subsequently be harvested.
- Upon completion of perfusion the animal carcass is wrapped, placed in a plastic bag, and placed in the freezer until it is disposed of in DLAR.

1.17.2 Fixatives

- 4% paraformaldehyde in saline or PBS
- 3% formaldehyde (freshly prepared from paraformaldehyde); 1.5% glutaraldehyde; 2.5% sucrose
- Formalin 10%
- Other fixatives should be included in your protocol

1.17.3 Safety Concerns

Formaldehyde is a colorless, highly toxic, and flammable gas at room temperature. It is a strong smelling chemical which is commonly used in research and medical laboratories as an aqueous solution. This document establishes procedures for the safe handling and use of formaldehyde, formalin, and paraformaldehyde solutions used in perfusions. Formalin is a 40% formaldehyde solution while paraformaldehyde is a polymerized form of formaldehyde that depolymerizes when heated. A 4% formaldehyde solution is typically used in animal perfusion. Note that 10% neutral buffered formalin is approximately 4% formaldehyde.

1.17.4 Health Effects

Formaldehyde can act as a sensitizing agent and is a known human carcinogen that is linked to nasal cancer and lung cancer. Acute exposure is highly irritating to the respiratory system and can cause headaches and eye and throat irritation at very low concentrations.

Signs of acute exposure: Nasal, throat, and pulmonary irritation.

Signs of chronic exposure: Headaches, rhinitis, drowsiness, respiratory impairment, kidney injury, pulmonary sensitization, and tissue damage. May also cause neuropsychological effects such as sleep disorders, irritability, altered sense of balance, memory deficits, loss of concentration, and mood alterations.

1.17.5 Regulatory Limits

The OSHA Formaldehyde standard (29CFR 1910.148) protects workers exposed to formaldehyde and apply to all occupational exposures to formaldehyde gas, its solutions, and materials that release formaldehyde. The permissible exposure limit (PEL) for formaldehyde is 0.75 parts per million (ppm) of air as an 8 hour time weighted average (TWA). The short-term exposure limit (STEL) is 2ppm maximum exposure over a 15 minute period. OSHA also defines an action level of 0.5ppm when calculated as an 8-hour TWA which requires increased monitoring and initiation of worker medical surveillance.

1.17.6 Air Monitoring

Area monitoring can be requested to assess potential exposures in the general laboratory work area. The monitoring is performed using a direct read instrument to give concentrations in room during perfusions. Personal monitoring can be requested to determine potential exposures for individual employees who work with formaldehyde. The monitoring is performed using a passive dosimeter that the employee wears in their breathing zone to quantify potential exposure. Air monitoring can be requested by contacting Environment, Health & Safety (EH&S).

1.17.7 Engineering Controls

Formaldehyde solutions used in perfusions should be used in a chemical fume hood, ducted biological safety cabinet, or downdraft table to achieve formaldehyde levels less than the TWA or STEL.

1.17.8 Personal Protective Equipment (PPE)

- Nitrile gloves, a lab coat, and safety glasses.
- When in rodent barrier facilities, PPE use must be consistent with the facility policy.

1.17.9 Waste Disposal

- Unused solutions of formaldehyde must be disposed of as a hazardous waste per University Hazardous Waste policies and procedures.

- Do not discard perfusion waste down the sink.

1.17.10 Accidents or Injuries

- If formaldehyde is splashed on an individual or in eyes, flush for 15 minutes with copious quantities of water and seek medical treatment.

1.17.11 Spill procedures

- Do not attempt to clean-up if you feel unsure of your ability to do so or if you perceive the risk to be greater than normal laboratory operations.
- If a small spill occurs rapidly absorb any liquid with absorbent pads or paper towels and place in a plastic bag with hazardous waste (red orange) label and store in a chemical fume hood prior to hand off to EH&S at regular hazardous waste pickup.

If a large spill occurs notify others in the area and evacuate room immediately. Call 911 (campus phone) or 631-632-3333 (cell phone) to request spill clean-up assistance.

1.18 Production of Subcutaneous Tumors in Rodents

1.18.1 Anesthesia

1) Anesthesia may be used for subcutaneous tumor inoculation but is not required.
2) Anesthetic Agents

   Mice → Isoflurane (Drop method or vaporizer)
   Ketamine (90-120 mg/kg) and Xylazine (5-10 mg/kg), IP

   Rats → Isoflurane (Drop method or vaporizer)
   Ketamine (60-75 mg/kg) and Xylazine 5-10 mg/kg), IP

3) The use of any other anesthetic agents must be listed in the IACUC application.

1.18.2 Anesthesia Monitoring

During the injection procedure the following parameters must be monitored at a minimum of 15-minute intervals:

- Respiratory rate
- Response to noxious stimulus (i.e. toe pinch)
- Spontaneous movement

1.18.2 Anesthesia Recovery Monitoring

1) During recovery from anesthesia, the following parameters must be monitored at a minimum of 15-minute intervals until the animal is ambulatory:
   - Respiratory rate
   - Movement
   - Ability to maintain sternal recumbency
2) To protect the animal from hypothermia (injectable anesthesia or if recovery is expected to be longer than 3-5 minutes) they should never be placed directly on metal surfaces. Animals should be placed on a water re-circulating heating blanket and/or covered well, or wrap them in a paper towel (while still allowing visible monitoring) to conserve body temperature.
3) It is estimated that animals will recover within 30-60 minutes (for injectable anesthesia).

1.18.4 Parameters for Tumor Production

1) The maximum tumor size is 2.0 cm in diameter. However, tumors may need to be harvested before this maximum size is reached depending on tumor location and growth pattern. Important factors for early tumor harvesting include the ability of the animal to move around the cage normally, reach food and water, and evidence of tumor infection or necrosis.
2) No more than two tumors may be inoculated per animal.
3) There is potential for contamination of transplantable tumors and tissue cell lines with a variety of murine pathogens (e.g., LCM, MHV, MVM). For this reason, all murine derived tumor and tissue cell lines are required to undergo IMPAC testing or PCR testing before their use in animals can begin. This requirement also pertains to animals that have been inoculated with these murine tissues at other facilities and are planned to be transferred to the DLAR. Contact the DLAR Veterinary staff for testing information. It is recommended that murine cell lines be checked periodically for pathogens.

1.18.5 Tumor Inoculation Procedure

1) A 23-25 gauge needle or 13 gauge trochar is preferred for tumor cell inoculation.
2) The back and flank are the approved sites for tumor placement. Any other location requires scientific justification in the IACUC application.
3) Cells are typically injected intradermally or subcutaneously.

1.18.6 Analgesia

None required

1.18.7 Adverse Effects

Potential adverse effects of subcutaneous tumor growth include:
- Erythema of the tumor or surrounding skin

- Ulceration, infection, bleeding and/or necrosis of the tumor
- Metastasis of the tumor leading to systemic signs such as weight loss, respiratory distress, diarrhea, lethargy, abdominal distension, rough hair coat(unkempt appearance).
- Self-mutilation due to pruritis (itching)
- Limited ability to move and/or reach food and water

1.18.8 Clinical Monitoring and Management

1) Post inoculation, animals must be monitored weekly until a palpable tumor nodule is present.
2) Once a nodule is present, the animals should be monitored at least three times per week for the potential adverse effects listed above.
3) If tumor growth is rapid, daily monitoring should include weekends and holidays.

1.18.9 Early Endpoints

1) Animals must be euthanized if the tumor becomes ulcerated, infected, necrotic, or bleeds.
2) If an animal, due to the size or location of tumor, cannot move normally or cannot easily reach food and water, they must be euthanized.
3) Mice with tumors larger than 2 cm in diameter must be euthanized(except see below).

1.18.10 Special consideration models

In some rare cases, the research protocol requires exceeding the 2 cm diameter early endpoint. Examples of this would include some mammary tumor models that affect the entire chain of mammary glands, or when the subject of the research is tumor metastasis that may not occur until the after the tumors have reached 2 cm. These exceptions must be stated and justified in the IACUC protocol, and the first 2 early endpoints list in Section I will still apply.

The Veterinary Staff will work with the Principal Investigator when evaluating these mice, but they will retain the final authority in determining if euthanasia is warranted.

1.19 Monoclonal Antibody Production in Laboratory Animals

Please consider In Vitro ascites as an alternative. The following guidelines are intended to eliminate, or reduce to a minimum, animal discomfort associated with the production of hybridomas.

Hybridomas, when grown in the abdominal cavity, will produce both a solid mass and ascitic fluid. As the volume of the abdominal cavity is limited, severe distension may occur which can lead to respiratory distress and gastrointestinal compromise. When ascites fluid is removed, the rapid loss of a large volume of fluid may cause hypovolemia, renal insufficiency

and edema. Any deviation from these guidelines requires specific justification in the IACUC protocol.

1) Weigh animal prior to initiating any procedures to obtain baseline weight and record.
2) Rodents may be primed once with a maximum dose of 0.2 ml pristane (2,6,10,14-tetramethylpentacane) from 10 days to 3 -4 weeks prior to inoculation with hybridoma cells. Preferably a volume of 0.1-0.2 mls should be utilized. Incomplete Freunds Adjuvant (IFA) may be substituted for pristane at the same volume. No anesthetic is required for pristane priming. Animals should be monitored at least 3 times weekly following priming.
3) Once animals are primed with pristane and the myeloma implantation begun, they should be observed at least once daily, including weekend and holidays, to ensure that excess bloating and respiratory distress does not occur. Ascites fluid should be tapped before these signs occur.
4) Withdraw ascites fluid through a 20-25 gauge needle attached to a syringe. With the mouse lying on its back, swab the abdomen with alcohol and insert the needle into the abdomen. Massaging the abdomen gently will facilitate fluid removal. Monitor and record weight every other day.
5) The maximum frequency of taps is every other day. The total number of taps should be determined by daily monitoring of the animals' health status. Animals exhibiting signs of anorexia, weight loss, hunched posture, ruffled fur, increased respiratory rate, lethargy, immobility or who are in lateral recumbency should be euthanized and have a final, terminal tap performed immediately.
6) In general, ascites fluid should not exceed 20% of normal body weight.
7) When possible, all animals should be euthanized within 28 days of hybridoma injection.
8) Hybridoma recipients must be histocompatible. Interspecies monoclonal antibody production must be scientifically justified in the IACUC application.

1.20 Polyclonal Antibody Production and Use of Freund's Adjuvant

The improper or unnecessary use of Freund's complete adjuvant may cause excessive inflammation and skin necrosis in laboratory animals during polyclonal antibody production. Before selecting Complete Freund’s adjuvant, please carefully consider the alternative use of another adjuvant (e.g., RIBI) or incomplete Freunds adjuvant.

You must review the MSDS form for Freund’s Adjuvant located at:
https://ehs.stonybrook.edu/resources/safety-data-sheet.php

The IACUC has developed the following Standard Procedures intended to eliminate, or reduce to a minimum, animal discomfort associated with the use of adjuvants. Any procedural deviation from these Standard Procedures requires specific justification in the IACUC protocol.

1.20.1 Sedation/Anesthesia

1) Sedation and/or anesthesia may be used for antigen injections and for blood withdrawal, but is not required.
2) Sedatives
   Rabbits – Acepromazine 1-2 mg/kg, IM or IV, performed by trained individual using a rabbit restraint device or with an assistant.
3) Anesthetic Agents

   Rabbits - Ketamine (35 mg/kg) and Xylazine (5 mg/kg), IM, can be supplemented with Isoflurane inhalation.

   Mice - Ketamine (40-120 mg/kg) and Xylazine (10 mg/kg), IP
   Pentobarbital (50 mg/kg), IP, Isoflurane inhalation

4) If other sedatives or anesthetic agents are to be used they must be listed in the IACUC application.

1.20.2 Anesthesia Monitoring

1) During the procedure the following parameters must be monitored at a minimum of 5-minute intervals:
   - Respiratory rate
   - Response to noxious stimulus
   - Spontaneous movement

1.20.3 Injection Procedure

1) The injection sites should be carefully shaved, removing all fur.
2) The site should then be cleansed with an appropriate antiseptic agent (betadine scrub, followed by an alcohol rinse) prior to injection.
3) Preparation of the inoculum:
   a) The inoculum should be free of extraneous microbial contamination.
   b) Millipore filtration of the antigen before mixing with the adjuvant is recommended.
   c) Acrylamide gels can cause animals additional pain. It is recommended that acrylamide gels not be included in the adjuvant preparation (e.g. elute the protein antigen from the acrylamide whenever possible). If acrylamide gel is used, clearly describe the pain medication you will use to alleviate the pain.
4) The inoculum, containing the antigen and adjuvant, should be divided into fractions so that no more than 0.1 ml is injected per site for rabbits and 0.05 ml for mice.
5) Injections should be subcutaneous. Investigators must scientifically justify immunization by any other route for the following reasons:
   - Intradermal injections may result in skin necrosis
   - Intramuscular injections may result in temporary or permanent

lameness and significant muscle necrosis
6) Footpad injections are highly discouraged and require strong scientific justification.
7) Intraperitoneal (IP) injections are only acceptable in mice

1.20.4 Antigen/Adjuvant Injection Parameters

1) Complete Freund's adjuvant should be used only for the first (priming) antigenic dose.
2) If incomplete Freund's adjuvant is used for the second and subsequent doses, an interval of 1 week should be allowed between doses.
3) Use of two or more doses of complete adjuvant must be scientifically justified in the IACUC application. If more than one dose must be used, an interval of at least 3 weeks should be allowed between doses.

1.20.5 Anesthesia Recovery Monitoring

1) During recovery from anesthesia, the following parameters must be monitored at a minimum of 15-minute intervals until the animal is ambulatory.
   - Respiratory rate
   - Movement
   - Ability to maintain sternal recumbency
2) To protect the animal from hypothermia they should be placed on a water re-circulating heating blanket, or covered well, to conserve body temperature. Never place animals on metal surfaces.
3) It is estimated that animals will recover within 30 minutes.

1.20.6 Analgesia

No analgesia is required for these procedures.

1.20.7 Blood Withdrawal Procedures

1) Rabbits should be bled from the marginal ear vein and mice should be bled either from the tail vein or from the saphenous vessels, depending on volume of blood collected.
2) The maximum volume of blood withdrawal is 5 ml/kg per week
   or 10 ml/kg, once every 14 days, for both species
3) Final blood collection (exsanguination) may be taken from the heart via intracardiac puncture. This procedure must be done under anesthesia and is a non-survival procedure.

1.20.8 Adverse Effects

1) Potential adverse effects of antigen/adjuvant inoculation include:
   - Erythema, swelling, ulceration, infection and/or necrosis of the injection sites

- Tenderness of injection sites (pain)
- Anorexia, lethargy, weight loss of greater than 15% of initial body weight or any signs of distress

2) Potential adverse effects of blood collection include:
   - Hypovolemia
   - Infection of the blood collection site
   - Hematoma formation

1.20.9 Clinical Monitoring and Management

1) Post inoculation, animals must be monitored at least twice weekly for the clinical signs listed above.
2) Post-bleeding, animals must be monitored once daily for 2 days.
3) If any of the signs listed above are seen, the investigator should contact the DLAR Veterinary staff so that appropriate medical treatment can be initiated.

1.20.10 Early Endpoints

Any animals with injection site lesions that are unresponsive to treatment or animals who have weight loss >15% initial body weight must be euthanized.

1.21 Standard Inveon (Ct/Pet/Spect) Imaging of Rats and Mice

1.21.1 Facility

Access to the Inveon Room is obtained by using the Lenel ID reader outside the ultrasound room. Access to the imaging room must be specifically requested in the DLAR office.

Only users who have been trained by either the Inveon representative or one of the designated training staff will be permitted to use the imaging machine. Training sessions can be scheduled with the training staff at mutually convenient times.

Scans for the protocol may be conducted by either trained users or a designated training staff member.

1.21.2 Inveon Room Equipment Use

All gas tanks must be secured properly in the anesthesia cart or in an approved stand. An extra oxygen tank will be available in case an empty tank needs to be changed during a scanning session. Advise the vet staff when a tank is emptied so it can be replaced promptly. Remember to turn off the oxygen tank when finished.

Each user is responsible for supplying their own isoflurane, which can be purchased from DLAR. The unused isoflurane can be drained from the machine at the end of the imaging session and returned to your bottle. See Vet staff for instructions, or to report any problems with the ultrasound or anesthetic machines.

The imaging machine and scan bed must be cleaned with a mild soap and dried after each use.

Do not leave animals in the imaging room overnight. Maximum isolation animals should be returned to the post imaging room (currently room 87). The conventional animals can be returned to their original room. However, if animals are still 'radioactive' at the time they are to be returned to the housing room, they should be taken to the 'hot' post-imaging room adjacent to the imaging room.

1.21.3 Anesthesia

Mice and rats must be anesthetized with either inhalational isoflurane gas or an alternative injectable anesthetic for the imaging sessions.

For inhalational anesthesia, the individual animal is first placed into the clear plastic induction box, then they are transferred to the appropriately sized scanning bed.

The nose of the animal is placed into the end of the nosecone. Make sure the anesthetic tubing inside the scan bed is not kinked.

The use of any anesthetic agent other than isoflurane must be identified in the IACUC application as an exception to the Standard Procedure.

1.21.4 Anesthesia Monitoring

Please list the length of the scanning procedure and describe the anesthetic monitoring (if any) that will be done in section H2 of the protocol.

1.21.5 Anesthesia Recovery Monitoring

To protect the animal from hypothermia they should never be placed on metal surfaces – place animals on a water re-circulating heating blanket, or wrap them in a towel (while still allowing visible monitoring) to conserve body temperature. Thermal packs can also be used. During recovery from anesthesia, the following clinical parameters must be monitored at a minimum of 5-minute intervals until the animal is ambulatory:

- Respiratory rate
- Movement

- Ability to maintain sternal recumbency (i.e. sit upright)

Animals should be left to recover in a cage without bedding to eliminate possibility of bedding blocking the airway if the animal crawls into the corners of the recovery cage. In addition, be alert to the possibility of cage mates injuring nonresponsive animals if housed together in recovery.

It is estimated that animals will recover within 3-5 minutes post scanning for inhalational anesthetic, 15-30 minutes for injectable.

1.21.6 Inveon Scanning Procedure

The limbs and/or torso of a rat or a mouse are taped to the appropriately sized scanning bed. An initial image/scout shot is taken to confirm proper positioning of the animal.

The animal is typically injected prior to the scan, via tail injection through a needle, butterfly or temporarily placed indwelling catheter.

The desired scan will be run (CT, CT/PET, CT/SPECT) according to the parameters entered into the Inveon.

After imaging, the scanning bed will be homed and the animal removed and placed into the anesthetic recovery cage.

1.22 Telemetry Unit Implantation in Rodents

1.22.1 Anesthesia

1) Anesthesia is required for surgical implantation of the telemetry unit and associated catheters and bio-potential leads.
2) Anesthetic agents – animals will be anesthetized with one of the following agents for this procedure.

   Mice - Ketamine (80-120 mg/kg) and Xylazine (5-10 mg/kg), IP
   Isoflurane (via vaporizer), delivered to effect.

   Rats - Ketamine (75 mg/kg) and Xylazine (5-10 mg/kg), IP
   Isoflurane via vaporizer, delivered to effect

3) Use of any other anesthetic agents must be identified in the IACUC application

1.22.2 Anesthesia Monitoring

Prior to and during the procedure the following parameters will be monitored at least every 5 minutes:

- Respiratory rate
- Response to noxious stimulus (toe pinch)
- Spontaneous movement

1.22.3 Anesthesia Recovery Monitoring

1) During recovery from anesthesia, the following clinical parameters must be monitored at least every minute until the animal is ambulatory:
   - Respiratory rate
   - Movement
   - Ability to maintain sternal recumbency
2) To protect the animals from hypothermia they will be placed on a water recirculating heating blanket, or covered well, to conserve body temperature. Animals should never be placed on metal surfaces.
3) It is estimated that animals will recover within 30-60 minutes post-operatively.

1.22.4 Surgical Procedures

Telemetry Unit Insertion Procedure – Femoral catheter/subcutaneous transmitter placement

1) Shave and disinfect the ventral abdomen and the inner thigh region on the side of proposed transmitter placement and drape surgical site. Disposable, adhesive drapes are preferred for rodents.
2) Open the skin over the femoral vessels.
3) Using blunt dissection, form a subcutaneous pocket up towards the area between the caudal edge of the ribcage and the most cranial extension of the knee's range of motion. Ideally, the subcutaneous pocket should be just large enough for the transmitter body to be inserted into the pocket, with the tissue snug, but not taut over the transmitter.
4) Once placed in the pocket, secure the transmitter housing by passing 5-0 suture through the tissues surrounding the pocket entrance and drawing together the entrance in a purse-string fashion.
5) Locate and expose femoral vessels. They are bundled together with the saphenous nerve and can be found between the abdominal wall and the branching point of the caudal epigastric artery and vein.
6) Carefully isolate the femoral artery from the femoral vein and saphenous nerve (~10 mm if possible). NOTE: Care must be taken not to damage the nerve during isolation of the artery to prevent hind limb paresis or paralysis.
7) Pass three lengths of 5-0 non-absorbable suture underneath the isolated artery section (proximal occlusion, artery ligature, and distal occlusion).

8) Irrigate the femoral artery with 2% lidocaine to dilate the vessel and prevent vasospasms.
9) Apply tension to the proximal and distal sutures to occlude blood flow and elevate the artery.
10) Pierce the artery with catheter introducer (bent-tip, 22g. needle) cranial to the distal occlusion suture and slip the catheter tip proximally into the vessel.
11) Temporarily release tension on the proximal occlusion suture and slide the catheter beyond it into the iliac region.
12) Advance the catheter beyond the iliac bifurcation until the pressure-sensing tip is situated in the abdominal aorta.
13) Tie the middle, ligature suture around the artery so that it seals the artery wall around the catheter stem. Release the proximal occlusion suture and observe for leakage. A small drop of veterinary tissue adhesive may be applied to catheter insertion point to seal the juncture.
14) Release the tension on the distal occlusion suture and use it to ligate the downstream section of the femoral artery. Once the ligature is knotted around the artery, tie the suture ends around the catheter stem to stabilize the preparation.
15) To keep the catheter in the proper orientation, suture the catheter stem to surrounding muscle tissue and loop the catheter subcutaneously to prevent kinking.
16) Close the incision with sutures or staples and seal the incision with sterile tissue adhesive to help prevent infection. Skin clips and non-absorbable suture materials should be removed 7-10 days post-operatively.
17) Administer the appropriate post-operative analgesic and monitor the animal for return of normal behaviors.

1.22.5 Telemetry Unit Insertion Procedure – Abdominal aorta catheter/ peritoneal cavity transmitter placement

This is the procedure of choice when use of femoral arteries is prohibited due to study needs and/or when accurate core body temperature is required. All surgical procedures must follow the Rodent Survival Surgery.

1) Shave and disinfect the ventral abdomen from the xiphoid process to the pelvis and drape surgical site. Disposable, adhesive drapes are preferred for rodents.
2) Make a 4cm midline abdominal incision for mouse(10 cm for rat) to allow good visualization of the peritoneal cavity from the renal arteries down to the aortic bifurcation.
3) Expose the contents of the abdomen using a spring retractor.
4) Hold back the intestines using saline moistened gauze sponges to allow good visualization of the descending aorta on the dorsal body wall.
5) Gently dissect the aorta from the surrounding fat and connective tissue using sterile cotton applicators.
6) Clear excess tissue from the aorta to allow for good hemostasis following catheterization.

7) Carefully insert an occlusion ligature (2-0 non-absorbable suture) between the aorta and the vena cava, just caudal to the left dorsal muscular branch. Form a loop under the aorta that will allow occlusion of blood flow. Care should be taken not to damage the vessel as this may result in thrombosis or fibrosis of the catheter tip later.
8) Restrict the blood flow using the proximal ligature to elevate the vessel in preparation for catheterization. NOTE: The aorta blood flow must not be restricted for greater than 3-4 minutes to avoid hind-limb paralysis due to ischemia
9) Puncture the aorta just cranial to the aortic bifurcation using a 21-gauge needle bent 90° at the beveled end. Make sure the concave surface of the bevel faces down, and do not place more than the beveled needle tip into the blood vessel.
10) Slide the tip of the catheter under the needle using the needle as a guide (or use a catheter introducer), and pass the catheter cranial until the entire thin-walled section is within the vessel
11) Thoroughly dry the puncture site and surrounding tissue to assure complete bonding of the tissue adhesive. Apply one drop of sterile tissue adhesive to the puncture site, and place a cellulose patch on the glue. Lift catheter to allow complete glue penetration.
12) Replace intestines into their original position and moisten with sterile saline.
13) Simultaneously close the body wall and secure the transmitter into place by closing the abdominal incision. Incorporate the "suture rib" on the telemetry unit into the closure using non-absorbable sutures (3-0 or 4-0) in a simple interrupted pattern.
14) Close the incision with sutures or staples and seal the incision with sterile tissue adhesive to help prevent infection. Skin clips and non-absorbable suture materials should be removed 7-10 days post-operatively.
15) Administer the appropriate post-operative analgesic and monitor the animal for return of normal behaviors.

1.22.6 Telemetry Unit Insertion Procedure – Carotid catheter/ subcutaneous transmitter placement

This procedure may be used as an alternate to catheter placement in the descending aorta or femoral artery, depending on study objectives. All surgical procedures must follow the procedures for Rodent Survival Surgery.

1) Shave and disinfect the ventral throat area and drape surgical site. Disposable, adhesive drapes are preferred for rodents.
2) Make a midline incision from the sternum to the jaw (about 2.5 cm). Retract the salivary glands to expose the muscles overlying the trachea.
3) Locate the carotid artery along the left side of the trachea and carefully isolate the vessel from the vagus nerve
4) Pass two lengths of non-absorbable suture material underneath the isolated section of artery.
5) Position one suture just proximal to the bifurcation of the external and internal carotid arteries and ligate the vessel.

6) Position the other suture close to the clavicle and apply tension to elevate the artery and occlude blood flow
7) Pierce the vessel just below the point of ligation with a bent-tipped 25g needle and insert the catheter. Advance the tip at least 2 mm so that the catheter notch is near the carotid bifurcation. Tie in the catheter using the two sutures.
8) Use blunt tipped dissecting scissors to form a subcutaneous pouch to hold the transmitter. The location of the pouch will depend on the species and vary from the neck (large animal) to the lateral flank (mouse). Insert the transmitter into the pouch and secure with sterile tissue adhesive.
9) Close the skin incision with sutures or staples. Skin clips and non-absorbable suture materials should be removed 10 – 14 days post-operatively.
10) Administer the appropriate post-operative analgesic and monitor the animal for return of normal behaviors.

1.22.7 Biopotential Lead Placement for ECG/EMG recording

This ECG/EMG procedure may also be performed for some studies following the placement of catheter and transmitter by one of the methods described above. All surgical procedures must follow the policy for Rodent Survival Surgery.

1) For ECG/EMG lead placement, the skin overlying the lead placement sites are shaved and disinfected when the animal is prepared for catheterization as described above. The ECG sites are the right cranial and left medial thorax; the EMG site is study dependent.
2) A small incision is made in the skin overlying the lead placement sites. The lead wires are tunneled subcutaneously to their placement sites and attached to the muscle using 4-0 non-absorbable sutures. In mice, the leads may simply be tunneled but not sutured to the muscle.
3) Close the incision with sutures or staples. Skin clips and non-absorbable suture materials should be removed 10-14 days post-operatively.
4) Administer the appropriate post-operative analgesic and monitor the animal for return of normal behaviors.

1.22.8 Equipment malfunctions/recovery

If the catheter, biopotential leads, or transmitter ceases to work, attempts will be made to diagnose the problem. If it can be determined that the malfunction can be corrected by simple adjustments to the positioning or the placement of the equipment, a subsequent surgery may be conducted to accomplish this. This will occur only if the additional survival surgery is deemed justified by the clinical veterinarian, after consultation with the principal investigator. A decision to perform recovery surgery will be made based on the value of the research animal due to historical data already obtained and the adequacy of the battery charge remaining on the transmitting unit. All surgical procedures must follow the for Rodent Survival Surgery. Following euthanasia, all equipment will be removed, cleaned with

enzymatic detergent, soaked overnight in 2% activated glutaraldehyde, and thoroughly rinsed in sterile saline before re-use. Non-functioning units or those with depleted batteries will be returned to the vendor for reconditioning.

1.22.9 Post-Operative Analgesia

Analgesia will be provided under the direction of the Clinical Veterinarian but will include at least a single post-operative dose of Buprenorphine (Mice – 2 mg/kg SQ, Rats – 0.01 - 0.05 mg/kg SQ). Additional doses will be administered as needed until the animal is not showing any signs of pain (inappetence, poor grooming, hunched posture).

1.22.10 Adverse Effects

Potential adverse effects from this procedure are minimal but may include:

- Anesthetic related respiratory distress
- Infection of the subcutaneous pocket, abdominal cavity or catheter insertion site
- Dehiscence of the surgical site
- Seroma formation around the transmitter
- Hind limb paresis or paralysis related to ischemia or nerve damage
- Hemorrhage due to leaking of the vessel around the catheter insertion site
- Post-operative pain as evidenced by:
    - Decreased activity
    - Decreased food and water intake
    - Rough hair coat, hunched posture
    - Weight loss
    - Vocalization when the incision is touched
    - Isolation of the individual from the group

Adverse effects can be prevented or limited by trained surgeons using aseptic surgical techniques and the proper choice of suture materials, as described above.

1.22.11 Clinical Monitoring and Management

1) Animals will be monitored daily until the skin sutures or staples are removed (7-10 days), and then once to twice weekly until completion of the study.
2) The attached Rodent Post-operative Monitoring Sheet can be used to assist investigators with pain assessment.
3) If any adverse effects are seen, the Clinical Veterinarian will be informed immediately and appropriate treatments administered. Animals not responding to treatment will be euthanized.

1.22.12 Early Endpoints

Animals that are showing signs of pain or distress including inappetance, hunched posture, poor grooming, weight loss of more than 15%, or difficulties locomoting will be removed from study and reported to the clinical veterinarian. If deemed untreatable, these animals will be euthanatized.

Animals showing swelling or discharge from the incision site, or dehiscence will be reported to the clinical veterinarian. If deemed untreatable, these animals will be euthanatized.

1.23 In Vivo Micro CT (uct) of Rodents

1.23.1 Anesthesia

1) Anesthesia is required for imaging using the uCT.
2) Anesthetic Agents - animals should be anesthetized with one of the following agents for this procedure:
   Mice - Ketamine (90-120 mg/kg) and Xylazine (5-10 mg/kg), IP
   Isoflurane (1-2.5%)

   Rats - Ketamine (60-75 mg/kg) and Xylazine 5-10 mg/kg), IP
   Isoflurane (1-2.5%)
3) Use of any other anesthetic agents must be identified in the IACUC application.

1.23.2 Anesthesia Monitoring

Please list the length of the scanning procedure and describe the anesthetic monitoring (if any) that will be done in section H2 of the protocol(IACUC application)

1.23.2 Anesthesia Recovery Monitoring

1) During recovery from anesthesia, the following clinical parameters must be monitored at a minimum of 5 minute intervals until the animal is ambulatory.
   - Respiratory rate
   - Movement
   - Ability to maintain sternal recumbency
2) To protect animals from hypothermia they should be placed on a water recirculating heating blanket, or well covered well, to conserve body temperature. Animals should never be placed on metal surfaces.
3) It is estimated that animals will recover within 30-60 minutes postoperatively

Scanning the animal
CT measurements will be taken both before initiation of an experimental protocol as well as during the experimental procedures. Each scan will take approximately 60-120 minutes per mouse which will be taken in the DLAR facility in the Psychology A Building. The radiation

dose of this scanner is relatively low. The local average dose per scan of 200 slices is approximately 25 mGy, compared to the LD50 (lethal dose at which 50% of mice die) which is orders of magnitude higher (2Gy).

Use of contrast agents: Iodinated contrast agents are used routinely both in the clinic and in the laboratory in doses ranging from 300mg/kg-9000mg/kg. Doses up to at least 2000mg/kg are free of detrimental side-effects and even doses as high as 9000mg/kg are non-lethal in rodents and do not cause pain. For some studies, iodinated contrast agent may be used in doses up to 5000 mg/kg in both mice and rats for imaging vessels and soft tissues. Other contrast agents are based on small peptides or nano-particles.

Please specify in the experimental methods section and substance administration sections of the protocol(IACUC application) exactly which contrast agents will be used for your scans.

Adverse Effects

1) Potential adverse effects for the procedures include:
   - Anesthetic related respiratory distress
   - Peritonitis secondary to trauma during the IP injection of contrast agent
2) Adverse anesthetic effects will be avoided by proper dosing and titration of anesthetic agents and close monitoring of animals during the anesthetic period. Only properly trained individuals will perform the IP injections.

Clinical Monitoring and Management

1) Animals should be monitored daily for two days following each scan.
2) If adverse effects are seen, the investigator should consult immediately with the DLAR veterinary staff regarding treatment options.

Early Endpoints

If animals show any signs of peritonitis including inappetance, hunched posture, or inactivity, they will be euthanized immediately.